COMPARATIVE PHYSIOLOGY OF SAPROPHYTIC AND PARASITIC FUNGI INHABITING SOIL

by

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COMPARATIVE PHYSIOLOGY OF SAPROPHYTIC AND PARASITIC FUNGI INHABITING SOIL.

A DISSERTATION
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES
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ABSTRACT

Studies were undertaken to compare certain aspects of the physiology of representative saprophytic and parasitic soil inhabiting fungi. Four fungi were studied in particular, two saprophytes *Trichoderma viride* Pers. ex Fries, and *Trichocladium asperum* Harz, and two parasites *Ophiobolus graminis* Sacc., and *Fomes annosus* Fries.

Comparisons were made with regard to tolerance to antibiotics, nutritional behaviour, and response to plant growth substances. The following conclusions were drawn:

1. The saprophytes were generally more tolerant to antibiotics than the parasites but exceptions to this were noted.
2. Differences in growth rate were found to be of a specific nature.
3. Study of pH and temperature requirements suggested that certain combinations of these two factors alone might favour the growth of at least one of the parasites (*O. graminis*) over the two saprophytes.
4. All four fungi were capable of some growth in synthetic media containing nitrate as the nitrogen source, but the two parasites showed a much greater preference for more complex forms of nitrogen than did the saprophytes.
5. *F. annosus* required thiamine and *O. graminis* required thiamine and biotin for growth on a synthetic medium containing either inorganic or organic nitrogen. Both saprophytes grew satisfactorily without added vitamins.

6. Differences in the ability of the fungi to use representative carbon sources were largely of an individual nature, but the parasites appeared better equipped to break down the more complex molecules.

7. Indoleacetic acid inhibited rather than stimulated all four fungi.

8. The parasitic fungi were typically inhibited by lower concentrations of indoleacetic acid than were the saprophytes.

9. The difference in indoleacetic acid inhibition probably resided in a greater ability on the part of the parasites to break down the molecule through enzyme action.

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INTRODUCTION.

The purpose of the investigations described here was to compare certain aspects of the physiology of saprophytic and parasitic soil inhabiting fungi and an attempt has been made to correlate the findings with the ecology of the fungi studied.

The soil is not homogeneous and the ability of fungi to colonize some of the many habitats it presents must be correlated with their physiological behaviour. Waksman (216) has classified the components of the soil into five main groups, namely: mineral particles, plant and animal residues, living systems, water and gases. An indication of the complexity of soil environments is only obtained when the variations which occur in each of these main groups is considered.

Many soil types have been recognized (168), differentiated by variations in size and chemical composition of the mineral particles. Locally the mineral constituents of the soil are augmented by the numerous break-down products of plant and animal remains. Living organisms varying in size from tree roots and small mammals to amoebae, fungi and bacteria contribute to the complexity of the soil both physically and chemically. Considerable variations are known to occur in the moisture content of the soil, and in the composition of the soil atmosphere.

The number of possible combinations of these variables is almost incomprehensible, but in addition each of these groups is influenced by the others, and also by external factors such as atmospheric pressure, light, temperature, rainfall and snowfall, fire, trampling and tillage. Here are mentioned only factors of which we have some knowledge; there may be many

more of which we know nothing.

To attempt to unravel some of these complexities for fungi may seem almost foolhardy especially when consideration is given to the large numbers and kinds of these organisms which are to be found in the soil.(10, 221).

Optimistically, however, for the purposes of the investigations to be described here the soil may be divided into two main microbial environments, the boundary between them being the outer surface of the living roots of higher plants. Fungi which can cross this boundary and can colonize the living tissue within the roots are referred to as parasites, those that do not are termed saprophytes. As with most distinctions in nature the two groups are not mutually exclusive, many fungi exhibit both saprophytic and parasitic ability. Typical parasites, however, such as the two to be considered here do not compete successfully in the soil and typical obligate saprophytes do not commonly invade living tissues.

From the ecological point of view there are, therefore, two main questions. Firstly, why do parasitic fungi appear to be confined in the soil to living roots, their saprophytic existence being a temporary one in the freshly dead tissue of their hosts? That success as a parasite would logically be increased by extended saprophytic survival has been pointed out by Bawden (4).

Secondly, why do obligate saprophytic soil fungi not invade living plant tissue? Since competition between micro-organisms for nutrients in the soil is believed to be intense (203), why is the ample reservoir in the plant root not tapped by these fungi?

The situation appears even more paradoxical when some of the

characteristics which have been attributed to the two groups are considered, particularly of the saprophytic group. These include for the latter group generally greater vigour manifested in greater rates of growth and sporulation, and ability to colonize a variety of substrates, or ability to decompose special substrates such as lignin (75), greater resistance to antibiotics and toxins in general and greater production of antibiotics (24); parasites on the other hand, are not believed to possess these qualities, at least to the same degree.

These conclusions appear to be drawn from scattered observations throughout the literature and surprisingly little relevant information is available concerning the representatives of the two groups. No comparative investigations appear to have been undertaken.

The investigations are reported under three main headings: 1, tolerance to antibiotics; 2, nutritional behaviour, and 3, response to plant growth substances.

THE FUNGI STUDIED.

Trichoderma viride Pers. ex Fries.

T. viride is one of the most commonly isolated soil saprophytes (10, 78) which probably explains the large number of references to this fungus in the literature. Surprisingly few references are to be found prior to the observations of Weindling (225) on the antagonism of this fungus to Rhizoctonia solani. Waksman (214) found species of Trichoderma

in all the soils he examined and mentions the frequency with which it had been reported by other investigators. Coleman (42) also listed T. koningi as one of the more common species found in the soil. Boswell (14), Boswell and Gover (15) and Boswell and Sheldon (16) found it to be present in acid soils, being isolated from there on filter paper soaked with nutrient solution. It has been recorded as a common, early recolonizer of sterilized soils (55, 129) and of fumigated soils (76, 138, 219, 220). Comparatively few papers deal with physiological studies of T. viride. These will be referred to subsequently where appropriate.

Confusion has arisen in the past over the identity of various forms of Trichoderma and several specific names appear in the literature. The situation was clarified by Bisby (9) when he demonstrated that from a single ascospore of Hypocrea rufa, imperfect cultures, showing all the variations formerly considered as specific differences, could be obtained. The green imperfect forms were, therefore, all classed as T. viride.

The isolate used, chiefly, in this study and referred to throughout the text as Trichoderma viride was obtained from one inch below the surface of a wheat field soil. It was isolated on agar plates containing cellulose as carbon source. This isolate grew rapidly on agar plates, sporulating abundantly in typical concentric rings. A second isolate was also used in some of the experiments with indoleacetic acid; this was found on the surface of an ergot sclerotium buried in the soil and will be referred to as T. viride (E). This isolate produced a dense white growth, grew comparatively slowly, sporulating much more sparsely, but again showing typical zonation.

The abundant references to the saprophytic ability of T. viride made

it a logical choice for study.

Trichocladium asperum Harz.

T. asperum, the second saprophyte used in this investigation, appears, from the paucity of the literature concerning it, to have been the subject of comparatively little interest. Gilman (78), who refers to it by the synonym Dicoccum asperum Corda, indicates that it has been recorded in Canada (10), Russia and the United States. It was readily isolated on soil plates containing cellulose, from the same soil sample as T. viride. It forms a thick dense type of growth both on solid and liquid media, producing large numbers of typical two-celled spores. The colour of the cultures was usually white at the periphery (on malt yeast dextrose agar) varying through shades of yellow and green within, and becoming almost black with age.

Hughes (104) describes and illustrates the genus Trichocladium.

Ophiobolus graminis Sacc.

O. graminis was selected for study as being one of the most typical root parasites. It infects cereals and a wide variety of wild and cultivated grasses (155), and causes the well known disease of wheat, 'Take-all'. O. graminis is found throughout the temperate regions of the world and is considered by Garrett (72) to rank second only to stem rust in the economic losses it causes to wheat growing. In view of its importance for wheat it is of interest that the first recorded description appears to have been from species of Agropyron and Cynodon (Saccardo (177)).

The life cycle and pathology of O. graminis have been the subject of considerable study. Garrett (72) reviews the pertinent literature.

The isolate of O. graminis used in this investigation was obtained

from wheat roots. Subsequent pathogenicity tests indicated that the fungus was able to produce typical disease symptoms in wheat seedlings.

In a consideration of the taxonomy of *O. graminis*, Arx and Olivier (3) suggested that it be transferred to a new genus *Gaeumannomyces* and called *G. graminis*. The old name has been used in this report, carrying, it is felt, more significance for those who have studied the biology of the fungus than the new one.

Fomes annosus Fries.

F. annosus has been recorded as a frequent cause of root-rot and heart-rot of conifers in the northern hemisphere (165). Some disagreement is to be found in the literature with regard to the parasitic status of this fungus. By some it has been regarded as a secondary parasite able to infect only previously invaded roots or cut stumps. Recent extensive investigations by Rishbeth (165, 166, 167), however, indicate clearly that on pines (*Pinus sylvestris* and *P. nigra* var. *calabrica*) at least, *F. annosus* is a true parasite, invading living roots of all ages, but only at points where contact has been made with a previously infected root. Spread of the fungus apparently did not occur through the soil, but did occur along the outer surface of roots; the saprophytic life of the fungus was restricted to that in roots which had died after infection.

A number of references are to be found in the literature to the physiology of this fungus and these will be referred to where pertinent.

The isolate used here was obtained from the Forest Pathology Laboratory, Science Service, Calgary, Alberta.

Several other fungi were used in this study but only to a limited

extent, and it is felt that a separate description here is not warranted.

GENERAL MATERIALS AND METHODS.

Although the particular methods used in the separate parts of the investigation will be considered with the experiments, certain general procedures apply throughout and are considered here.

SOURCE OF MATERIALS.

Water.

The water used throughout was distilled in a regular copper still and collected in glass carboys. No further purification was carried out.

Agar.

The agar used to solidify certain media was Difco 'Bacto- Agar'.

Chemicals.

The sources of the various chemicals were as follows: British Drug Houses - asparagine, manganese sulphate; California Foundation - adenine, adenosine, calcium d-pantothenate, i-inositol, niacinamide, thiamine hydrochloride, pyridoxine hydrochloride, indoleacetic acid, 2,4-dichlorophenoxyacetic acid, maleic hydrazide; Eastman Kodak Company - pectin (citrus), 2,4-dichlorophenol; Fischer Scientific Company - D-glucose, sucrose, potassium dihydrogen phosphate, magnesium sulfate, ferric chloride, zinc sulphate, sodium chloride, potassium hydroxide, boric acid, calcium chloride; Nutritional Biochemicals - D-fructose, D-mannose, D-galactose, L-sorbose, L-arabinose, D-arabinose, D-ribose, D-xylose, maltose, lactose, melibiose, cellobiose, inulin, starch, cellulose, biotin, p-aminobenzoic acid, indolebutyric acid, malt extract, yeast extract, yeast hydrolysate;

Mallinkrodt - ferric sulphate, ammonium sulphate, potassium nitrate; Sigma - 2,4,6-trichlorophenoxyacetic acid.

In each case the highest grade obtainable from the sources indicated was used.

GLASSWARE.

All glassware used in experiments was of "Pyrex" brand glass, and was cleaned in hot detergent solution ("Calgon" or "Alconox") and rinsed first in tap water (6x) and then in distilled water (6x).

STERILIZATION.

Sterilization of dry glassware was carried out by dry heat at 250°C for several hours.

Careful attention was paid to the sterilization of media and other solutions.

There have been several reports in the literature of the undesirable effects of steam sterilization of culture media. Thus Englis and Hanahan (53) found that on autoclaving glucose with phosphates for 30 minutes at 15 lbs. pressure considerable conversion to fructose occurred together with the development of a brown colour in the solution. Hill and Patton (102) showed that autoclaving tryptophane with sugars reduced the growth of *Streptococcus faecalis*, and McKeen (130) found that autoclaving glycine with dextrose made media toxic to *Phytophthora fragariae*. Ramsey and Lankerford (160) reported that heating of glucose resulted in the production of some compound stimulatory to growth of *Lactobacillus fermentii*. The nature of the compound was not determined but it was believed to be a product of glucose and phosphate.

Obviously in physiological and biochemical studies changes during autoclaving in the identity of the compounds used is most undesirable.

Nevertheless, alternative methods of sterilization (such as filtration) are usually unsatisfactory especially where large volumes are required.

If changes occur in media as a result of heating then it is likely that the extent of such changes will vary with the amount of heat supplied. This being so it would also be expected that great care would be exercised in controlling the temperature and the length of time for which heating is carried out during sterilization procedures, but unfortunately this is seldom the case. Most authors follow the standard procedures adopted before the process of steam sterilization was understood. Thus Riker and Riker (164) and Lilly and Barnett (124) both recommend autoclaving for 15 or 20 minutes at 15 lbs. steam pressure, and many authors do not bother to state the conditions of autoclaving that were used.

Underwood (208) pointed out that the steam pressure is no indication of sterilizing conditions and that the important factor is temperature, which is seldom recorded in the literature. He also observes that unless all the air is removed from the sterilizing chamber, temperatures at the bottom of the chamber sufficient for sterilization may never be reached. Thus in all critical work in the present investigation an autoclave was used in which the temperature of the steam exhaust leaving the base of the chamber was indicated.

The amount of heat required for sterilization depends upon the volume of material to be sterilized. Underwood (208) presents data which illustrate this and points out that the more moist the conditions the more rapidly does sterilization occur. In the investigation reported here, media and other organic materials were sterilized either in flasks or tubes containing 25, 20 or 15 ml. of liquid; sterilization for no more than 8

minutes at a temperature of 121°C, as indicated by the exhaust steam, was found to be satisfactory for such quantities. Sterilization under these conditions did not result in any discolouration of any of the solutions and, it was hoped, little alteration of the chemical constituents.

CULTURES.

Cultures of the fungi studied were maintained on slants of malt-yeast-dextrose agar in test tubes. After satisfactory development they were transferred to a cold room at 0°C.

WEIGHING.

The weighing of all chemicals for which accuracy was required was performed on a Mettler analytical balance, reading to five places of decimals (of grams).

The same balance was employed for the determination of mycelial weights in physiological experiments. The mycelium was collected on filter papers (previously dried and weighed) and the gross dry weights determined. The dry weights of mycelium were obtained by difference. Drying was carried out in an electric oven at 75°C for 24 hours; after removal from the oven the filter papers were placed in a desiccator to cool before weighing.

RECORDING OF DATA.

The data from all the experiments where growth was determined are of two kinds, either growth in diameter on an agar plate or mycelial weights.

The first are recorded here only as the average value of 3 or 4 cultures. This was considered desirable to save space and sufficient as very close agreement usually was found between plates with the same treatment.

The results of the experiments where mycelial weights were obtained are reported in full, the considerable variation inherent in this type of experiment necessitating this for the sake of completeness. There seems to be little agreement in the literature as to how this kind of data should be treated. In the investigation reported here either 4, 5 or 6 flasks were harvested for each treatment. Norkrans (148)and Vining and Taber (209), for example, use similar numbers, but many other investigators do not, notably Lilly and Barnett (e.g. 126), who harvest mycelium from two flasks and take the mean weight. Very few investigators carry out any statistical analyses of this kind of datum. Here the data for the majority of experiments have been subjected to an analysis of variance such as was carried out by Taber (195) and only values significantly different at the 1% level have been accepted. It should be pointed out that additional conclusions might be drawn from some of the work if the treatment of data had been the same as that of the majority of workers.

PART I. INFLUENCE OF SELECTED ANTIBIOTICS ON THE GROWTH OF TRICHODERMA VIRIDE, TRICHOCLADIUM ASPERUM, OPHIOBOLUS GRAMINIS AND FOMES ANNOSUS.

INTRODUCTION.

That antibiotics play an important role in the inhibition of micro-organisms in natural soil is a matter that still awaits positive proof. Waksman (216) regards antibiotic production as a laboratory process requiring enriched culture media and the development of high yielding strains. It is true that antibiotic production by a micro-organism in a soil medium has only been demonstrated a) when the soil has been sterilized to remove competitive micro-organisms (54, 56, 85, 88, 99, 101, 192, 246), b) when the soil has been sterilized and supplemented with organic matter (29, 56, 86, 88, 91, 100, 101, 192, 246, 247), and c) in unsterilized soil supplemented with large quantities of organic matter (88, 246, 248, 250).

However, the methods available for the determination of antibiotics in soil are likely to be successful only when production is general throughout the sample tested; in addition there are certain factors which tend to limit the amounts of an antibiotic which can be detected. For example, many antibiotics are strongly adsorbed on clay colloids reducing the amount of 'free antibiotic' in the soil solution (85, 88, 109, 134, 183), and chemical and biological breakdown has also been reported in some cases (29, 85, 109, 185, 218). Further, under natural conditions, considering the complexity of the soil population, it is unlikely that any

one antibiotic would be formed anywhere but in restricted localities. It is possible, as Waksman states, that enriched conditions are required for antibiotic production, but such conditions do occur locally in the soil too, in, on and around fragments of organic matter such as the debris of higher plants. It is in such localized environments that greatest microbial activity is likely to occur and here, also, antibiotic production would probably be greatest. This is the view of Brian (24) and some supporting evidence is found in the work of Wright (249, 250), who has demonstrated the production of gliotoxin and other antibiotics in the coats of wheat seeds and in wheat straw buried in the soil.

It is, therefore, reasonable to assume that among fungi which pass some portion of their life history in the soil, tolerance to antibiotics will have considerable survival value. This raises the question whether parasitic fungi, which in general are relatively unsuccessful saprophytes, have a lower tolerance to antibiotics than saprophytic fungi. Garrett (73) suggests that this is the case. Butler (37, 38, 39) observed that of two cereal root-rot fungi, *Curvularia ramosa* and *Helminthosporium sativum*, the former was a much more vigorous saprophyte. This difference could be explained by the much greater resistance of *C. ramosa* to a range of antifungal antibiotics. Brian (24) also considers parasitic fungi to be in general more sensitive to antibiotics. A brief investigation was therefore undertaken to compare the response of *Trichoderma viride*, *Trichocladium asperum*, *Ophiobolus graminis* and *Fomes annosus* to various concentrations of some selected antibiotics.

MATERIALS AND METHODS.

ANTIBIOTICS.

Acti-dione,

(supplied by the Upjohn Company, Kalamazoo, Michigan.)

Acti-dione has attracted considerable interest, particularly since it was used successfully as early as 1948 in the control of powdery mildew of bean (58), and has subsequently been put into commercial use for the control of cherry leaf spot and certain turf grass diseases.

Acti-dione is produced by certain strains of *Streptomyces griseus* (229, 231). Its chemical structure is beta-(2-(3,5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl)-glutarimide (116), from which its generic name cycloheximide is derived. Whiffen (229,230) reported that acti-dione exhibited remarkable specificity in its action on micro-organisms, having little effect on bacteria but being particularly effective against fungi. Ford et al. (60) give a review of the pertinent literature concerning acti-dione.

Gliotoxin,

(supplied by the Prairie Regional Laboratory, Saskatoon.)

Gliotoxin was the name given by Weindling (227) to the toxic substance observed by him to be produced by *Trichoderma viride* (226) during his classic studies of the parasitism of *T. viride* on *Rhizoctonia solani* (225, 226). Brian (21) and Brian and Hemming (28) also demonstrated independently the production of gliotoxin by *T. viride*.

Gliotoxin is described by Brian (23) as being both antibacterial and antifungal and has been shown to be produced by at least four fungi -

T. viride, Aspergillus fumigatus, Penicillium terlikowskii and P. cinerascens.

Griseofulvin,

(sample supplied by Dr. P. W. Brian).

Griseofulvin was apparently first isolated as a metabolic product of Penicillium griseofulvum by Oxford, Raistrick and Simonart in 1939 (153). The same substance was isolated by Brian et al. (25) from P. janezewskii and called the 'curling factor', because it caused peculiarities in the development of the hyphae of Botrytis allii and other fungi. That the two substances were identical was demonstrated by Grove and McGowan (92) and Brian et al. (27). Brian (22) reported that it is produced by at least three species of Penicillium (P. griseofulvum, P. patulum and P. janezewskii).

Griseofulvin attracted attention not only because of its antibiotic properties but also because of the morphological effects it produced in fungal hyphae. It was noted (22) that griseofulvin was without effect on cellulose-walled fungi (Oomycetes) but was effective on fungi having chitinous walls, and the suggestion was made that griseofulvin was active in fungi in the same way that indoleacetic acid was active in higher plants.

Patulin. (Clavatin, clavacin, claviformin, expansine.)

(Obtained from the California Corporation for Biochemical Research.)

Patulin was isolated by Anslow et al. in 1943 (2) as a metabolic product from two fungi, Penicillium patulum Bainer and P. expansum (Link) Thom. Wiesner (234) and Waksman et al. (217) reported the production of clavacin by Aspergillus clavatus and this was demonstrated to be

identical with patulin (8). It has since been demonstrated as a product of a number of fungi, including Penicillium expansum, P. claviforme, P. urticae, P. melinii, P. divergens, P. terrestre, P. novae-zeelandiae, Aspergillus clavatus, A. giganteus, A. terreus and Gymnoascus sp. (23).

Patulin is antibacterial and also antifungal, in the latter case showing considerable specificity of action.

Patulin has been produced synthetically (242).

Trichothecin,

(supplied by the Prairie Regional Laboratory, Saskatoon.)

The name trichothecin was applied to an antifungal metabolic product of Trichothecium roseum by Freeman and Morrison (63). The same authors (64, 65) isolated the substance and determined its biological and chemical properties. It was reported to be highly antifungal but not markedly antibacterial. The behaviour of trichothecin in the soil was studied by Hessayon (99, 101).

All five antibiotics have been the subject of some detailed investigations, all are produced by soil fungi, and some of them have been demonstrated as products of more than one organism. Hence it was considered that they would be good representatives of this particular class of substances.

Only acti-dione was soluble in water in useful amounts. Acetone was used as a solvent for the other four. In addition to being a good solvent acetone had the additional advantage of a low boiling point, much of it being dissipated during autoclaving.

BASIC MEDIUM.

The basic medium used in the antibiotic studies was a rich organic medium on which all four fungi grew particularly well. It was made up as follows:

Malt extract	5 grams
Yeast extract	5 grams
Glucose	15 grams
Agar	17 grams

Distilled water to make 1 litre

INOCULUM.

Cultures were grown at room temperature[*] in Petri dishes containing 15 ml. of the basic medium per dish. Plugs 5 m.m. in diameter were cut from the periphery of the cultures where possible and used to inoculate the experimental media.

PREPARATION OF EXPERIMENTAL MEDIA.

The experimental media consisted of the basic medium in which the antibiotics were incorporated in concentrations of 0, 0.1, 1.0, 5.0, 10.0, 50.0, and 100.0 p.p.m., except in the cases of gliotoxin and trichothecin where the highest concentrations used were 50.0 and 25.0 p.p.m. respectively.

Solutions of the antibiotics were made up at 10 times the strength required for each treatment. Acti-dione was dissolved in water and the others were dissolved in the following concentrations of acetone: gliotoxin 75%; griseofulvin 50%; patulin 25%; trichothecin 25%. The

[*] 22 - 25° C.

antibiotic solutions were then diluted with 9 parts of basic medium to give the required antibiotic concentrations. One part of water was added to the control for acti-dione and where acetone was used as a solvent this was incorporated in all treatments at the same concentration. The bottles of media containing acetone were stood in boiling water for 10 minutes to permit evaporation of acetone and the volume was made up with distilled water. No deleterious effects on the growth of the fungi due to residual acetone could be detected.

Fifteen ml. portions of the heated media were transferred to test tubes and autoclaved for 8 minutes. The contents of the tubes were then poured into Petri dishes and allowed to solidify. Twelve plates for each concentration of each antibiotic were prepared.

INOCULATION AND INCUBATION.

Three plates of each treatment were inoculated with each fungus, using the inoculum described above.

The cultures were incubated at room temperature in diffused daylight from a northern exposure. Growth of the fungi was recorded as the diameter of the colonies in millimetres, and measurements were usually made at approximately 24 hour intervals.

RESULTS.

The data obtained for the growth of the four fungi are presented in Tables 1, 2, 3, 4 and 5. The tables contain both the means from the three cultures per treatment and also the percentage growth for a

particular treatment based on the control at 100%. The latter permits a more direct comparison between species and antibiotics, and eliminates such factors as inherent differences in growth rate between the various fungi which should be considered separately.

The values for the percentage growth in the presence of 10 p.p.m. of each of the five antibiotics have been used for the graph Figure 1. Most of the important differences are illustrated at this concentration.

ACTI-DIONE. (TABLE 1)

T. viride was retarded by all concentrations above 0.1 p.p.m. and was strongly inhibited by 10.0 50.0 and 100.0 p.p.m. Good recovery was observed, and it was apparent that considerably higher concentrations would be required to inhibit this fungus over a longer period of time.

T. asperum was not inhibited to the same degree as T. viride as is indicated by the percentage figures (100.0 p.p.m. reduced growth to approximately 40% of the control), although the total surface growth of T. asperum was considerably less. However, if initial inhibition was less than with T. viride there was certainly no indication of any adaptation to acti-dione.

O. graminis was more severely inhibited by acti-dione than by any of the other antibiotics. The lowest concentration, 0.1 p.p.m., was definitely inhibitive and there was not a trace of growth at 5.0 p.p.m. In addition even at the concentrations where growth occurred there was no apparent adaptation.

F. annosus was also strongly inhibited by acti-dione but to a lesser extent than O. graminis. Growth did not occur above 10.0 p.p.m. where it commenced only after 96 hours, but there were indications that some

TABLE 1. EFFECT OF ACTI-DIONE ON THE GROWTH OF TRICHODERMA VIRIDE, TRICHOCLADIUM ASPERUM, OPHIOBOLUS GRAMINIS AND FOMES ANNOSUS.

a) TRICHODERMA VIRIDE.

ACTI-DIONE	GROWTH AS DIAMETER OF FUNGUS COLONY IN MILLIMETRES.								GROWTH AS PER CENT OF CONTROL.		
p.p.m.	TIME (hrs.)								TIME (hrs.)		
	24	48	72	84	96	120	144	168	24	48	72
0	9.3	52.0	85.0	– [1]					100.0	100.0	100.0
0.1	10.3	50.6	85.0	–					110.8	97.3	100.0
1.0	8.3	43.3	85.0	–					89.2	83.3	100.0
5.0	7.3	38.0	81.6	–					78.5	73.1	96.0
10.0	3.6	28.6	61.6	79.0	–				38.7	55.0	72.5
50.0	0	5.0	20.0	27.0	28.3	51.0	68.3	–	0	9.6	23.5
100.0	0	2.0	9.6	16.0	24.6	34.3	46.6	59.0	0	3.8	11.3

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[1] Plate completely filled.

TABLE 1 (CONTINUED).

b) TRICHOCLADIUM ASPERUM.

ACTI-DIONE	GROWTH AS DIAMETER OF FUNGUS COLONY IN MILLIMETRES.							GROWTH AS PER CENT OF CONTROL.						
p.p.m.	TIME (hrs.)							TIME (hrs.)						
	24	48	72	96	120	144	168	24	48	72	96	120	144	168
0	3.3	6.0	8.6	14.3	19.3	26.3	33.6	100.0	100.0	100.0	100.0	100.0	100.0	100.0
0.1	3.3	6.0	8.3	14.3	18.0	25.0	30.3	100.0	100.0	96.5	100.0	93.3	95.1	90.2
1.0	2.3	5.6	7.6	14.0	17.6	23.0	30.0	69.7	93.3	88.4	97.9	91.2	87.5	89.3
5.0	2.6	6.6	8.0	14.0	17.6	23.6	29.6	78.8	110.0	93.0	97.9	91.2	89.7	88.1
10.0	2.0	5.0	7.0	12.3	15.0	21.6	26.6	60.6	83.3	81.4	86.0	77.7	60.1	79.2
50.0	0	3.0	4.0	7.0	8.6	12.0	16.6	0	50.0	46.5	49.0	44.6	45.6	49.4
100.0	0	2.0	4.0	7.3	8.3	10.6	12.0	0	33.3	46.5	51.1	43.0	40.3	35.7

TABLE 1 (CONTINUED).

c) *OPHIOBOLUS GRAMINIS*.

ACTI-DIONE	GROWTH AS DIAMETER OF FUNGUS COLONY IN MILLIMETRES.						GROWTH AS PER CENT OF CONTROL.					
p.p.m.	TIME (hrs.)						TIME (hrs.)					
	48	72	96	120	144	168	48	72	96	120	144	168
0	13.3	23.6	37.6	48.0	63.5	76.5	100.0	100.0	100.0	100.0	100.0	100.0
0.1	8.6	17.6	29.0	36.6	46.6	55.6	64.7	74.6	77.1	78.3	73.4	72.7
1.0	0	3.3	6.4	7.3	8.3	8.3	0	14.0	16.8	15.2	13.1	8.5
5.0	0	0	0	0	0	0	0	0	0	0	0	0

TABLE 1 (CONTINUED).

d) FOMES ANNOSUS.

ACTI-DIONE	GROWTH AS DIAMETER OF FUNGUS COLONY IN MILLIMETRES.							GROWTH AS PER CENT OF CONTROL.					
p.p.m.	TIME (hrs.)							TIME (hrs.)					
	24	48	72	96	120	144	168	24	48	72	96	120	144
0	4.6	15.0	27.0	42.0	53.6	66.3	-[1]	100.0	100.0	100.0	100.0	100.0	100.0
0.1	3.3	10.0	24.6	40.0	51.0	61.3	75.0	71.7	66.7	91.1	95.2	95.2	92.5
1.0	2.3	8.0	13.6	24.6	32.6	41.6	52.3	50.0	53.3	50.4	58.6	60.8	62.7
5.0	0	0	2.3	4.0	6.0	7.6	8.6	0	0	8.5	9.5	11.2	11.5
10.0	0	0	0	0	3.0	4.0	4.6	0	0	0	0	5.6	6.3
50.0	0	0	0	0	0	0	0	0	0	0	0	0	0

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[1] Plate completely filled.

adaptation may have occurred.

Both O. graminis and F. annosus were much more susceptible to actidione than either of the two saprophytes.

GLIOTOXIN. (TABLE 2)

Inhibition of T. viride, T. asperum and O. graminis by gliotoxin was not particularly great and the percentage values are similar for concentrations up to 10.0 p.p.m. O. graminis was initially more inhibited by 50.0 p.p.m. but showed progressive adaptation to it.

F. annosus was quite strongly inhibited, 50.0 p.p.m. causing a considerable lag in the onset of growth; some adaptation probably occurred.

TABLE 2. EFFECT OF GLIOTOXIN ON THE GROWTH OF *TRICHODERMA VIRIDE*, *TRICHOCLADIUM ASPERUM*, *OPHIOBOLUS GRAMINIS* AND *FOMES ANNOSUS*.

a) *TRICHODERMA VIRIDE*.

GLIOTOXIN	GROWTH AS DIAMETER OF FUNGUS COLONY IN MILLIMETRES.				GROWTH AS PER CENT OF CONTROL		
p.p.m.	TIME (hrs.)				TIME (hrs.)		
	24	41	48	72	24	41	48
0	14.0	44.3	59.6	-[1]	100.0	100.0	100.0
0.1	14.6	45.0	60.6	-	104.3	103.9	101.7
1.0	16.3	47.0	62.6	-	116.4	106.1	105.0
5.0	13.0	44.0	59.6	-	92.9	99.3	100.0
10.0	11.3	39.6	55.6	-	80.7	89.4	93.3
50.0	9.0	36.0	51.6	-	64.3	81.3	86.6

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[1] Plate completely filled.

TABLE 2 (CONTINUED).

b) TRICHOCLADIUM ASPERUM.

GLIOTOXIN	GROWTH AS DIAMETER OF FUNGUS COLONY IN MILLIMETRES.					GROWTH AS PER CENT OF CONTROL				
p.p.m.	TIME (hrs.)					TIME (hrs.)				
	24	48	72	96	120	24	48	72	96	120
0	6.0	10.3	15.6	21.0	26.6	100.0	100.0	100.0	100.0	100.0
0.1	5.6	11.3	17.0	21.3	27.0	93.3	109.7	109.0	101.4	101.5
1.0	6.0	10.6	17.0	22.3	27.6	100.0	102.9	109.0	106.2	103.8
5.0	6.0	10.0	15.6	19.6	25.3	100.0	97.1	100.0	93.3	95.5
10.0	5.3	10.0	15.3	19.6	25.0	88.3	97.1	98.1	93.3	94.0
50.0	4.0	8.6	13.6	17.6	21.6	66.6	83.5	87.2	83.8	81.2

TABLE 2 (CONTINUED).

c) OPHIOBOLUS GRAMINIS.

GLIOTOXIN	GROWTH AS DIAMETER OF FUNGUS COLONY IN MILLIMETRES.					GROWTH AS PER CENT OF CONTROL.				
p.p.m.	TIME (hrs.)					TIME (hrs.)				
	24	48	72	96	120	24	48	72	96	120
0	6.0	14.3	25.3	38.3	49.6	100.0	100.0	100.0	100.0	100.0
0.1	6.3	14.6	27.3	36.0	47.6	105.0	102.1	107.9	94.0	96.0
1.0	7.3	14.6	23.3	35.3	51.0	121.6	102.1	92.1	92.2	102.8
5.0	5.6	14.0	22.6	33.6	55.0	93.3	97.9	89.3	87.7	90.7
10.0	5.6	14.6	21.3	32.3	41.6	93.3	102.1	84.2	84.3	83.9
50.0	2.6	8.3	14.0	26.3	37.0	43.3	58.0	55.3	68.7	74.6

TABLE 2 (CONTINUED).

d) FOMES ANNOSUS.

GLIOTOXIN p.p.m.	GROWTH AS DIAMETER OF FUNGUS COLONY IN MILLIMETRES. TIME (hrs.) 48	72	96	120	GROWTH AS PERCENT OF CONTROL TIME (hrs.) 48	72	96	120
0	10.6	22.3	37.0	52.0	100.0	100.0	100.0	100.0
0.1	8.6	21.3	35.6	49.3	81.1	95.5	96.2	94.8
1.0	8.3	18.3	31.3	43.3	78.3	82.1	84.6	83.3
5.0	4.3	11.6	25.3	30.6	40.5	52.0	68.4	58.8
10.0	2.0	9.3	19.0	29.0	18.9	41.7	51.4	55.8
50.0	0	6.0	15.3	20.3	0	26.9	41.4	39.0

GRISEOFULVIN. (TABLE 3)

All concentrations above 0.1 p.p.m. inhibited growth of T. viride to some degree; at 100.0 p.p.m. growth was only 10% of the control. There was no indication of any adaptation to this antibiotic, growth rates for a particular concentration remaining more or less constant. It was also noted that the zonation which is typical of this fungus was suppressed by griseofulvin.

T. asperum was less inhibited than T. viride but again all concentrations of griseofulvin showed some effect on growth. T. asperum gave no indication of adaptation to the antibiotic. It was of interest to note that in addition to the percentage growth the actual extent of growth of T. asperum at 50.0 and 100.0 p.p.m. was initially greater than that of T. viride, in spite of the latter's typically much higher growth rate.

O. graminis was generally less inhibited by griseofulvin than was T. viride, as indicated by the percentage figures; it also showed very definite adaptation to all concentrations, which was not true of either T. viride or T. asperum.

The response of F. annosus differed from that of O. graminis in that there was apparently no adaptation to the antibiotic and that it was only slightly inhibited by concentrations up to 50.0 p.p.m. - much less than T. viride and to about the same extent as T. asperum. At 50.0 and 100.0 p.p.m. there was a marked reduction in growth, but not to the extent that occurred in T. viride.

TABLE 3. EFFECT OF GRISEOFULVIN ON THE GROWTH OF *TRICHODERMA VIRIDE*, *TRICHOCLADIUM ASPERUM*, *OPHIOBOLUS GRAMINIS* AND *FOMES ANNOSUS*.

a) *TRICHODERMA VIRIDE*.

GRISEOFULVIN	GROWTH AS DIAMETER OF FUNGUS COLONY IN MILLIMETRES.								GROWTH AS PER CENT OF CONTROL.		
p.p.m.	TIME (hrs.)								TIME (hrs.)		
	24	43	48	62	68	93	117	150	24	43	48
0	15.0	50.0	62.6	-[1]					100.0	100.0	100.0
0.1	15.6	51.3	61.6	85.0	-				104.0	102.6	98.4
1.0	10.0	34.0	41.0	60.0	70.3	-			66.7	68.0	65.5
5.0	8.3	22.0	29.6	43.6	51.3	-			55.3	44.0	47.3
10.0	7.0	19.0	26.3	38.6	44.6	73.0	-		46.6	38.0	42.0
50.0	3.0	8.6	10.0	15.0	18.0	28.6	41.0	54.0	20.0	17.2	16.0
100.0	1.6	5.3	6.3	8.0	10.6	16.3	21.3	29.6	10.7	10.6	10.1

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[1] Plate completely filled.

TABLE 3 (CONTINUED).

b) TRICHOCLADIUM ASPERUM.

GRISEOFULVIN	GROWTH AS DIAMETER OF FUNGUS COLONY INMMILLIMETRES.						GROWTH AS PER CENT OF CONTROL.					
p.p.m.	TIME (hrs.)						TIME (hrs.)					
	24	48	68	93	117	150	24	48	68	93	117	150
0	6.0	10.0	12.3	13.3	18.3	24.6	100.0	100.0	100.0	100.0	100.0	100.0
0.1	5.6	10.0	12.3	12.6	16.6	24.3	93.3	100.0	100.0	94.7	90.7	98.8
1.0	5.3	9.6	12.0	12.6	15.6	24.0	88.3	96.0	97.6	94.7	85.2	97.6
5.0	5.0	9.3	12.0	12.0	15.3	23.0	83.3	93.0	97.6	90.2	83.6	93.5
10.0	5.0	9.3	11.3	11.0	14.3	22.0	83.3	93.0	91.9	82.7	78.1	89.4
50.0	4.3	7.3	8.3	10.0	13.0	18.6	71.7	73.0	67.5	75.2	71.0	75.6
100.0	3.6	5.6	6.6	8.0	11.3	14.6	60.0	56.0	53.7	60.2	61.7	59.4

TABLE 3 (CONTINUED).

c) OPHIOBOLUS GRAMINIS.

GRISEOFULVIN	GROWTH AS DIAMETER OF FUNGUS COLONY IN MILLIMETRES.						GROWTH AS PER CENT OF CONTROL.					
p.p.m.	TIME (hrs.)						TIME (hrs.)					
	24	48	68	93	117	150	24	48	68	93	117	150
0	3.0	15.0	22.0	31.3	44.0	62.0	100.0	100.0	100.0	100.0	100.0	100.0
0.1	2.0	14.0	26.6	32.0	43.3	61.6	66.7	93.3	120.9	102.2	98.4	99.4
1.0	1.3	8.0	12.0	19.6	28.6	43.0	43.3	53.3	54.6	62.6	65.0	69.4
5.0	1.0	4.3	8.6	13.6	23.6	41.3	33.3	28.7	39.1	43.5	53.6	66.6
10.0	1.0	4.3	8.6	15.0	22.6	34.3	33.3	28.7	39.1	47.9	51.3	55.3
50.0	0	2.6	5.0	10.0	14.0	20.3	0	17.3	22.7	32.0	31.8	32.7
100.0	0	2.3	4.0	5.3	9.0	13.6	0	15.3	18.2	16.9	20.5	21.9

TABLE 3 (CONTINUED).

d) *FOMES* *ANNOSUS*.

GRISEOFULVIN	GROWTH AS DIAMETER OF FUNGUS COLONY IN MILLIMETRES.					GROWTH AS PER CENT OF CONTROL				
p.p.m.	TIME (hrs.)					TIME (hrs.)				
	48	68	93	117	150	48	68	93	117	150
0	13.0	21.3	34.3	47.3	66.0	100.0	100.0	100.0	100.0	100.0
0.1	12.6	20.6	35.3	47.0	64.6	96.9	96.7	102.9	99.4	97.9
1.0	12.3	21.0	33.3	46.3	64.6	94.6	98.6	97.1	97.9	97.9
5.0	11.0	19.6	33.0	43.3	58.6	84.6	92.0	96.2	91.5	88.8
10.0	10.6	20.6	29.6	38.6	55.0	81.5	96.7	86.3	81.6	83.3
50.0	4.0	6.3	12.0	15.0	19.6	30.8	29.8	35.0	31.7	29.7
100.0	2.0	4.0	5.6	7.3	12.0	15.4	18.8	16.3	15.4	18.2

PATULIN. (TABLE 4)

T. viride was not appreciably inhibited by concentrations of patulin below 50.0 p.p.m. Definite inhibition occurred at 50.0 and 100.0 p.p.m. and at these concentrations there was some indication of adaptation.

Patulin inhibited T. asperum only slightly even at 100.0 p.p.m.

O. graminis was inhibited by all concentrations of patulin used, no growth occurring at 100.0 p.p.m. and only a small amount at 50.0 p.p.m. At concentrations of 10.0 p.p.m. and less there was very marked adaptation. Hyphal strands were produced at the higher concentrations.

The pattern of inhibition of F. annosus was similar to that of O. graminis but was somewhat less in degree; adaptation was again very strong. Enebo (52) found an extract from Penicillium claviforme (believed to be patulin) to be more active against F. annosus than extracts from several other fungi.

TABLE 4. EFFECT OF PATULIN ON THE GROWTH OF *TRICHODERMA VIRIDE*, *TRICHOCLADIUM ASPERUM*, *OPHIOBOLUS GRAMINIS* AND *FOMES ANNOSUS*.

a) *TRICHODERMA VIRIDE*.

PATULIN	GROWTH AS DIAMETER OF FUNGUS COLONY IN MILLIMETRES.						GROWTH AS PER CENT OF CONTROL.		
p.p.m.	TIME (hrs.)						TIME (hrs.)		
	24	43	48	62	68	93	24	43	48
0	14.6	49.6	61.0	–[1]			100.0	100.0	100.0
0.1	16.0	50.6	62.6	–			109.6	106.7	102.6
1.0	16.3	51.3	62.0	–			111.6	103.4	101.6
5.0	15.3	50.6	61.6	–			100.8	102.0	101.0
10.0	14.0	48.6	59.0	85.0	–		95.9	98.0	96.7
50.0	9.6	38.3	46.6	70.0	83.0	–	65.8	77.2	76.4
100.0	3.6	24.6	31.0	50.0	58.6	–	24.7	49.6	50.8

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[1] Plate completely filled.

TABLE 4 (CONTINUED).

b) TRICHOCLADIUM ASPERUM.

PATULIN	GROWTH AS DIAMETER OF FUNGUS COLONY IN MILLIMETRES.						GROWTH AS PER CENT OF CONTROL.					
p.p.m.	TIME (hrs.)						TIME (hrs.)					
	24	48	68	93	117	150	24	48	68	93	117	150
0	6.0	10.0	13.3	13.3	17.0	25.0	100.0	100.0	100.0	100.0	100.0	100.0
0.1	6.0	10.0	12.3	12.3	16.0	24.0	100.0	100.0	92.5	92.5	94.1	96.0
1.0	5.3	10.0	12.6	13.0	15.6	24.0	88.3	100.0	94.7	97.7	91.5	96.0
5.0	5.3	10.0	12.0	12.3	15.3	24.0	88.3	100.0	90.2	92.5	90.0	96.0
10.0	5.0	10.0	11.3	13.3	15.3	23.6	83.3	100.0	85.0	100.0	90.0	94.4
50.0	5.0	8.6	11.3	12.0	16.0	24.0	83.3	86.0	85.0	90.2	94.1	96.0
100.0	4.0	7.6	10.6	12.0	16.3	24.0	80.0	76.0	79.7	90.2	95.9	96.0

TABLE 4 (CONTINUED).

c) OPHIOBOLUS GRAMINIS.

PATULIN	GROWTH AS DIAMETER OF FUNGUS COLONY IN MILLIMETRES.						GROWTH AS PER CENT OF CONTROL					
p.p.m.	TIME (hrs.)						TIME (hrs.)					
	24	48	68	93	117	150	24	48	68	93	117	150
0	4.0	15.3	24.6	36.3	47.6	64.3	100.0	100.0	100.0	100.0	100.0	100.0
0.1	3.0	14.3	21.0	34.3	47.0	64.3	75.0	93.5	95.4	94.5	98.7	100.0
1.0	2.3	12.6	20.0	29.6	43.6	59.3	57.5	62.4	81.3	81.5	91.2	92.2
5.0	1.3	8.6	13.0	24.6	34.6	51.6	32.5	56.2	52.8	67.8	72.7	80.2
10.0	1.3	5.6	11.0	18.0	29.6	43.6	32.5	36.6	44.7	49.6	62.2	67.8
50.0	0	2.0	3.6	5.0	5.3	8.3	0	13.1	14.6	13.7	11.1	12.9
100.0	0	0	0	0	0	0	0	0	0	0	0	0

TABLE 4 (CONTINUED).

d) *FOMES ANNOSUS*.

PATULIN	GROWTH AS DIAMETER OF FUNGUS COLONY IN MILLIMETRES.					GROWTH AS PER CENT OF CONTROL.				
p.p.m.	TIME (hrs.)					TIME (hrs.)				
	48	68	93	117	150	48	68	93	117	150
0	12.0	21.3	36.6	49.0	67.0	100.0	100.0	100.0	100.0	100.0
0.1	13.3	22.6	34.6	49.0	66.6	110.8	106.1	94.5	100.0	99.4
1.0	11.6	20.6	32.3	48.0	66.3	96.6	96.7	88.3	98.0	99.0
5.0	8.6	18.0	31.3	43.3	60.6	71.7	84.5	85.5	88.4	90.4
10.0	7.0	16.0	27.3	41.6	63.3	58.3	75.1	74.6	84.9	94.5
50.0	2.0	8.0	11.3	24.3	37.0	16.7	37.6	30.9	49.6	55.2
100.0	0	0	0	0	0.3	0	0	0	0	0

TRICHOTHECIN. (TABLE 5)

Trichothecin was quite inhibitory even at 1.0 p.p.m. to *T. viride* and prevented growth for 24 hours at 25.0 p.p.m. There was, however, good recovery.

T. asperum was quite strongly inhibited by trichothecin; initially almost 50% reduction of growth occurred at 25.0 p.p.m. This was also the only antibiotic to which *T. asperum* showed a clear-cut adaptation.

O. graminis did not grow in the presence of 25.0 p.p.m. of trichothecin but at 0.1, 1.0 and 5.0 p.p.m. was somewhat less inhibited than was *T.viride* and was rather similar to *T. asperum*. Over all concentrations up to 25.0 p.p.m. the surface growth of *O. graminis* was greater than that of *T. asperum*. There was some adaptation to 5.0 and 10.0 p.p.m.

All concentrations of trichothecin inhibited *F. annosus*. No growth occurred at 25.0 p.p.m., growth was prevented for 96 hours at 10.0 p.p.m. and for 72 hours at 5.0 p.p.m. Growth was noticeably reduced compared with that of the other three fungi considered. Some adaptation probably occurred at 1.0 p.p.m.

TABLE 5. EFFECT OF TRICHOTHECIN ON THE GROWTH OF *TRICHODERMA VIRIDE*, *TRICHOCLADIUM ASPERUM*, *OPHIOBOLUS GRAMINIS* AND *FOMES ANNOSUS*.

a) *TRICHODERMA VIRIDE*.

TRICHOTHECIN	GROWTH AS DIAMETER OF FUNGUS COLONY IN MILLIMETRES.					GROWTH AS PER CENT OF CONTROL.		
p.p.m.	TIME (hrs.)					TIME (hrs.)		
	24	41	48	72	96	24	41	48
0	16.3	48.3	64.3	-[1]		100.0	100.0	100.0
0.1	15.6	46.6	61.0	-		95.7	96.5	94.9
1.0	10.0	39.0	54.3	-		61.4	80.8	84.5
5.0	5.6	31.0	45.0	-		34.4	64.2	70.0
10.0	1.6	23.6	34.6	71.3	-	9.8	48.9	53.8
25.0	0	17.6	25.6	49.3	74.0	0	36.4	39.8

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[1] Plate completely filled.

TABLE 5 (CONTINUED).

b) TRICHOCLADIUM ASPERUM.

TRICHOTHECIN p.p.m.	GROWTH AS DIAMETER OF FUNGUS COLONY IN MILLIMETRES. TIME (hrs.)					GROWTH AS PER CENT OF CONTROL. TIME (hrs.)				
	24	48	72	96	120	24	48	72	96	120
0	5.6	12.0	18.0	21.3	27.3	100.0	100.0	100.0	100.0	100.0
0.1	6.0	12.3	18.0	22.0	28.3	107.1	102.5	100.0	103.3	103.6
1.0	5.0	10.6	15.6	31.0	27.0	89.3	88.3	86.6	98.6	98.9
5.0	4.3	8.0	13.6	19.0	25.6	76.8	75.0	75.6	89.2	93.8
10.0	3.6	8.0	12.6	18.3	24.0	64.3	75.0	70.0	85.9	87.9
25.0	3.0	7.6	11.3	17.0	22.3	53.6	63.3	62.8	79.8	81.7

TABLE 5 (CONTINUED).

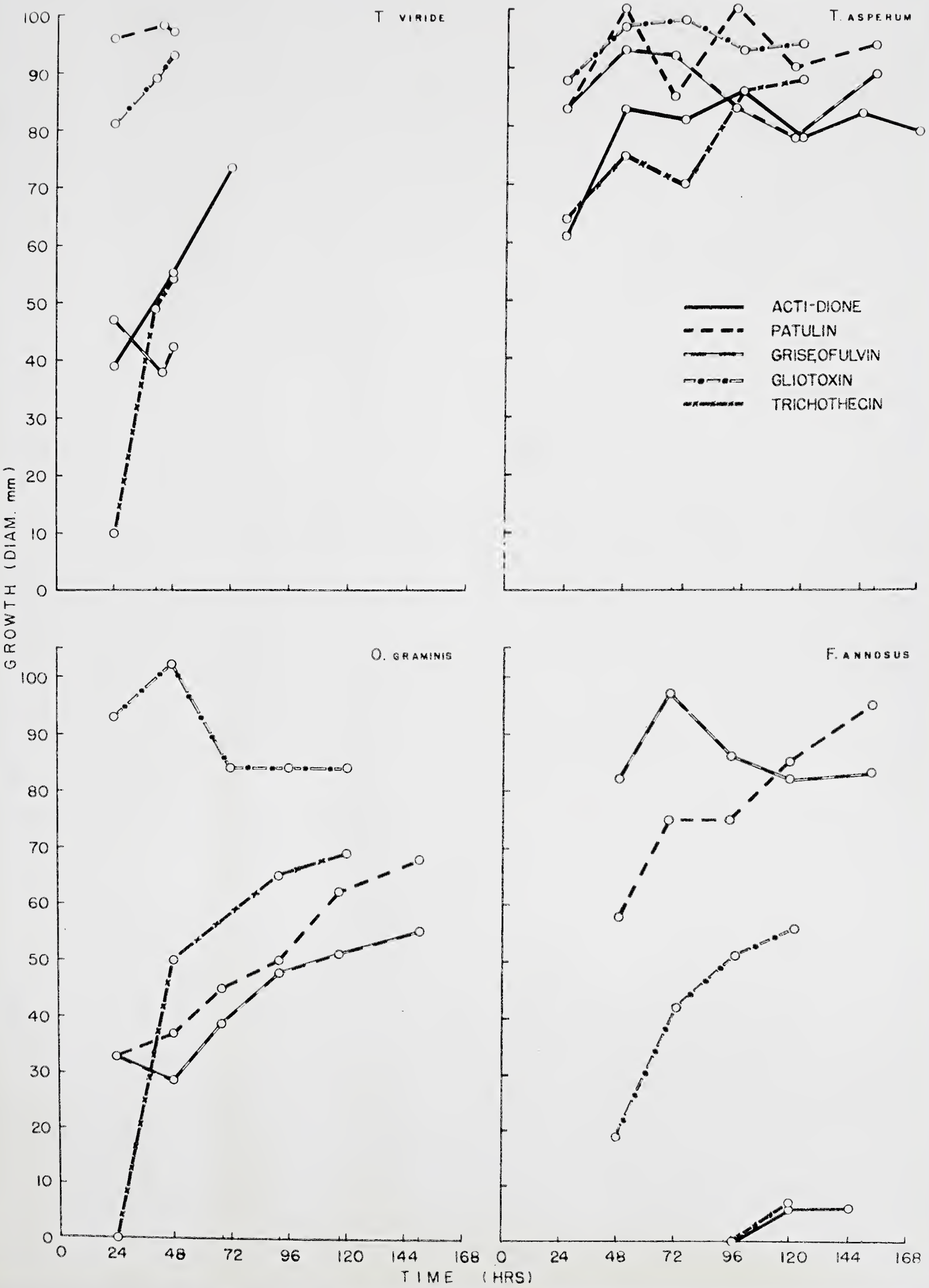
c) *OPHIOBOLUS GRAMINIS*.

TRICHOTHECIN	GROWTH AS DIAMETER OF FUNGUS COLONY IN MILLIMETRES.				GROWTH AS PER CENT OF CONTROL.			
	TIME (hrs.)				TIME (hrs.)			
p.p.m.	24	48	96	120	24	48	96	120
0	5.3	14.6	40.3	52.0	100.0	100.0	100.0	100.0
0.1	6.3	15.6	38.6	51.3	118.9	106.8	89.8	98.7
1.0	6.0	15.6	35.6	47.0	113.2	106.8	88.3	90.4
5.0	3.0	10.0	27.3	40.0	56.6	68.5	67.7	76.9
10.0	0	7.3	26.3	35.6	0	50.0	65.3	68.5
25.0	0	0	0	0	0	0	0	0

TABLE 5 (CONTINUED).

d) FOMES ANNOSUS.

TRICHOTHECIN	GROWTH AS DIAMETER OF FUNGUS COLONY IN MILLIMETRES.				GROWTH AS PER CENT OF CONTROL.			
p.p.m.	TIME (hrs.)				TIME (hrs.)			
	48	72	96	120	48	72	96	120
0	10.0	18.0	35.6	49.3	100.0	100.0	100.0	100.0
0.1	8.0	17.0	33.0	46.0	80.0	94.4	92.7	93.3
1.0	2.6	7.3	15.6	23.0	26.0	40.5	43.8	46.7
5.0	0	0	3.3	5.6	0	0	9.3	11.4
10.0	0	0	0	3.3	0	0	0	6.7
25.0	0	0	0	0	0	0	0	0


T. VIRIDE
T. ASPERUM
O. GRAMINIS
F. ANNOSUS
ACTI-DIONE
PATULIN
GRISEOFULVIN
GLIOTOXIN
TRICHOTHECIN
GROWTH (DIAM. mm)
TIME (HRS)
0
10
20
30
40
50
60
70
80
90
100
0
24
48
72
96
120
144
168

DISCUSSION AND CONCLUSIONS.

If a clear distinction with regard to antibiotic tolerance between the two saprophytes and two parasites used in this study had been found then it would have been good evidence in favour of the opinion of Garrett (73) and Brian (24) that antibiotics exert a selective action in the soil in favour of the saprophytes. That absolutely clear-cut differences were not observed does not, of course, preclude the possibility that in a majority of other comparisons such selectivity would be the rule. However, the number of exceptions recorded here make unconditional acceptance of Garrett's view unwise until definite supporting evidence is obtained.

Garrett bases his opinion on a report by Brian (22) in which the growth response of a number of fungi to griseofulvin was recorded. However, examination of the data reported by Brian does not reveal an obvious difference between saprophytes and parasites as Garrett suggests. For example, *Armillaria mellea* and several species of *Botrytis* were strongly inhibited. *Verticillium albo-atrum* and *Helminthosporium avenae*, however, were much less susceptible than *T. viride*, *Neurospora sitophila* and species of *Mucor* and *Absidia*. Garrett apparently also failed to observe that none of the Oomycetes tested were susceptible and these included species of *Pythium* and *Phytophthora*. Surprisingly Brian (24) who had not observed in his own data the phenomena which Garrett (73) had discovered, endorses and enlarges on the opinion of Garrett with evidence almost as slender.

In the investigations reported on here, it may be stated that

T. asperum showed superior resistance to antibiotics in practically all cases, but did not demonstrate any marked ability to adapt itself to inhibitory concentrations. T. viride was proportionately more susceptible to griseofulvin than either parasite and in addition was unable to adapt to this antibiotic as was O. graminis. The latter also showed little more susceptibility to gliotoxin than did either of the saprophytes.

It was of particular interest to note that with few exceptions the two parasites showed good adaptation to the antibiotics, superior to that shown by T. asperum and frequently better than that by T. viride. This illustrates the importance of collection of data over as long a period as possible, and it would have been interesting to study this phenomenon through a series of successive transfers on antibiotic-containing media.

In addition it should be mentioned that in comparing antibiotics determination of actual inhibitory concentrations are the criteria usually used. However, it is possible that frequently under natural conditions an organism may be subjected to less than completely inhibitory concentrations. When such is the case the problem of distinguishing between saprophytes and parasites is still more difficult. Occasionally at such concentrations the two parasites studied are less inhibited than the saprophytes, while being more strongly inhibited at higher concentrations. In the present studies this is true in a comparison of O. graminis and T. viride with 1.0 and 5.0 p.p.m. of trichothecin and of F. annosus and T. viride at the lower concentrations of griseofulvin.

Thus in conclusion it may be stated that although among fungi which inhabit the soil, resistance to antibiotics would be of greatest advantage to the soil saprophytes which are unable to invade living tissues, and

that a comparative lack of resistance on the part of parasites would serve as a general explanation of why they are usually unsuccessful competitors in the soil, evidence has not been obtained which supports this unconditionally. Tolerance to antibiotics may be a primary factor for survival in certain cases and under certain conditions, but under other conditions it may be of minor importance, and it would seem premature to regard it as necessarily a major factor of distinction between saprophytes and parasites.

PART II. INFLUENCE OF VARIOUS NUTRITIONAL FACTORS ON THE GROWTH OF TRICHODERMA VIRIDE, TRICHOCLADIUM ASPERUM, OPHIOBOLUS GRAMINIS AND FOMES ANNOSUS.

INTRODUCTION.

To state that all living matter requires an external supply of energy is axiomatic. It follows that the availability of energy sources will influence the distribution of living organisms. For higher plants (autotrophs) sunlight is the universal energy source. Fungi, which are heterotrophic, must obtain the energy required for living processes from complex compounds in their immediate external environment. As such chemical compounds are exceedingly numerous and exhibit such differences in degree of complexity, and as they also have a localized discontinuous distribution in space, it is reasonable to conclude that their availability and type will be a factor of prime importance in the ecological distribution of fungi.

Thus there is ample justification for the study of the nutritional behaviour of saprophytic and parasitic fungi. It may be anticipated that any specific requirement of a fungus not provided by a particular environment will prevent colonization. Also the presence of certain compounds used by a portion of a mixed population of fungi such as occur in the soil will tend to favour that portion over the remainder of the population. Unfortunately, an explanation of fungal ecology on a strictly nutritional basis is an over-simplification; as in the case of higher plants other factors are of importance. These include physical

factors, such as temperature, moisture and pH, and inorganic and organic inhibitors (of which antibiotics, the subject of Part I of this report, are examples); such organic inhibitors are probably of considerable importance in the resistance of most plants to the majority of fungi as suggested, for example, by the work of Virtanen (210), Virtanen et al. (213) and Turner (207).

The importance of nutrient availability for the distribution of both parasitic and saprophytic fungi has been considered by many investigators and is implicit in the reports of many others. Thom and Morrow (201) divided the soil fungi into two groups, those capable of breaking down animal and plant remains, and those which were able to survive on the residual products of such decomposition. Burges (33) classified the soil inhabiting fungi into two groups, the root infecting fungi and the obligate soil saprophytes. The latter he subdivided into 'sugar fungi' and 'humus fungi', according to the substrate relationships. Garrett (74) was probably the first to regard the parasitic soil fungi as a separate substrate group. He is not specific as to what particular substrates are involved in the invasion of the living host, but points out that this ability to invade a living root in advance of the saprophytic flora which invades the dead tissue gives the parasite a tremendous advantage from the point of view of survival. Lewis (123) formulated a theory of parasitism mainly on the basis of nutritional requirements, which was subsequently modified by Garber (70) to incorporate the effects of inhibitory compounds which may occur in plants.

GENERAL CONSIDERATIONS OF MATERIALS AND METHODS USED IN NUTRITIONAL STUDIES.

In these studies growth has been taken as a criterion of the utilization of nutrients. If it is assumed that growth is the only important outcome of nutrition then this criterion is satisfactory. This has been the assumption of the majority of workers who have carried out physiological studies with fungi. Steinberg (188, 191), Robbins and Kavanagh (171), Foster (61), Hawker (97) and Lilly and Barnett (124) summarize many examples of work of this kind. It should be pointed out, however, that there is a considerable amount of detailed work in the field of microbiology as a whole which demonstrates that utilization of a particular nutrient may not always result in growth. In fact, several methods of investigation of microbial physiology are based upon this fact. Such, for example, are the replacement culture methods described by Foster (61). In this technique metabolism of a particular compound is followed in the absence of growth of the fungus. The metabolism of glucose has, for example, been studied in this way and although under 'normal' cultural conditions metabolism of this substance would result in growth, in replacement cultures it is metabolized to various breakdown products which are again released into the medium. Such replacement cultures are usually maintained in the non-growing condition by eliminating nitrogen or some essential metabolite from the culture medium. Thus growth under a particular set of conditions may not be a criterion of utilization of a particular nutrient but only of the presence of other factors essential for the utilization of that nutrient.

This drawback to the use of growth as a criterion should serve to temper the dogmatic interpretation of results obtained from nutritional experiments and may serve to explain some of the differences apparent in the results of various investigators.

Various methods have been evolved for the determination of fungus growth experimentally, none of which is completely satisfactory.

Probably the simplest method is by measurement of surface growth of colonies growing on solid media. This is the only method which can be applied to fungi growing on solid substrates such as soil or wood. In the laboratory growth on media solidified with agar is usually measured in this way. This may be carried out in Petri dishes where the radius, diameter or area of the colony is measured, or in growth tubes of the type designed and used by Ryan et al. (176) where linear growth is measured.

This method has several disadvantages, the most obvious of which is that no account is taken of the density of the fungal growth. An attempt has been made by Worley (245) to take into consideration the tangential as well as linear growth of fungus mycelium, but this still does not incorporate the vertical growth and the method is complex and seldom used. If radial or linear growth was always proportional to the growth in the other two dimensions for a given fungus, that is if each fungus had a characteristic colony density on an agar surface, the disadvantage might not be so great, but unfortunately, the constituents of the substrate often influence tangential and vertical growth to a greater degree than linear growth. This was demonstrated in the present investigations in an attempt to determine growth on different carbon sources. The data

for Trichocladium asperum are given in Table 6.

TABLE 6. COMPARATIVE GROWTH OF TRICHOCLADIUM ASPERUM ON A SOLIDIFIED MEDIUM WITH AND WITHOUT A CARBON SOURCE.

	MEDIUM AND GLUCOSE	MEDIUM ALONE
DIAMETER OF COLONY IN MILLIMETRES AFTER 72 HOURS	15.0	22.0

Here the control medium consisted entirely of mineral salts and vitamins, $(NH_4)_2HPO_4$ being the nitrogen source. The data would indicate that better growth occurred in the absence of a carbon source than in its presence. The type of growth in the control medium consisted of very fine spreading surface mycelium of the type referred to by Hawker (97) as starvation-type growth, and in this case the diameter of the colony is most obviously not a satisfactory measure of growth.

Determination of the dry weight of fungus colonies on agar plates have been made by Day and Hervey (50) but these authors found that the agar could not be removed from the mycelium without heating and that in this process a significant amount of soluble material was lost from the hyphae.

A second objection often made to the use of agar in nutrient media is that it frequently contains physiologically active elements (170). However, in modern preparations impurities are reduced considerably and this objection is largely overcome.

Apart from these objections there are several points in favour of the use of agar cultures. Firstly, most fungi grow on solid substrates under natural conditions and the appearance of colonies on solid media are more typical than those on liquid. Cultures on solid media are also easily handled, can be moved from place to place and may be examined readily. Observations can be made on a single colony for a long period of time, and data on growth can be recorded at required intervals; replication of a series of plates is a simple matter.

Brancato and Golding (19) have recently discussed the validity of the agar-plate method for measuring fungus growth. They concluded that the method was satisfactory for the factors which they studied, namely: influence of temperature, concentration of the medium, relative humidity, reaction of the medium, size of spore inoculum, thickness of substrate, and atmosphere in the culture plate. They point out that replicate colonies show very close agreement, and that a characteristic growth rate is rapidly attained. Observations made during the investigations to be reported on below are in agreement with these conclusions.

The main alternative method of determining growth in culture is the one employing liquid media, either still or shaken, from which mycelium can be removed and the dry weight determined. Lilly and Barnett (124) state that this is the method of choice for precise work, and in fact have used it to a large extent in their published work. The chief drawback to this method is that from each culture flask only one set of data can be obtained, the total growth. To determine growth rates, which are of greatest value for comparison of different organisms, a large number of flasks needs to be prepared and all but the most simple experiment

becomes impracticable. A second disadvantage is that whilst almost all fungi can make some growth on solid media some fungi make much poorer growth on liquid media. Thus the use of liquid culture may put some fungi at a considerable disadvantage in a comparative study.

Foster (61) discusses the two methods of liquid culture (still or shaken) at some length, concluding that there are very considerable advantages in favour of the shaken cultures, chiefly due to factors of aeration and mixing of the contents of the medium, resulting in a much more homgeneous type of growth.

In the nutritional studies recorded below both solid and liquid media were used in different experiments depending upon the advantages which either method appeared to offer in each particular case. Thus solid media were employed in the study of general growth rates, the influence of temperature on growth rate and general studies on nitrogen and vitamin requirements. Liquid media were judged to be more satisfactory in the determination of pH requirements, due to the liquification of agar at low pH, and for the study of the utilization of different carbon compounds where measurement of growth on agar was unsatisfactory, as indicated earlier (Table 6).

The liquid cultures were not shaken, but only a small volume of medium was added per culture flask (20 ml. per 200 ml. Erlenmeyer) in order to permit maximum aeration.

In addition, the inoculum, consisting of a plug 5 m.m. in diameter cut from the edge of an agar culture, was placed on the bent tip of a glass rod which passed through the cotton bung and held the inoculum at the surface of the medium, according to the method described by

Converse (43), see figure 8.

Where possible the inoculum was grown on a medium corresponding to that used in the experiment for which it was intended. This was not always possible, for example in the experiments with carbohydrates where the choice of inoculum medium is indicated in the description of each experiment.

Incubation was carried out in the dark at room temperature (except in the temperature studies).

BASIC MEDIUM.

Although certain constituents of the medium were the subject of experimental study the composition was basically the same throughout, and was made up of the following: carbon source; nitrogen source; major mineral sources; minor mineral sources; vitamin supplement.

Carbon source.

In all experiments except where the utilization of different carbon sources was the subject of the experiment, D-glucose was the source of carbon. Usually glucose was used at a rate to give 5.0 grams carbon per litre of complete medium (i.e. 12.51 grams); where a larger quantity was used this is indicated.

The choice of glucose was based upon the fact that more fungi have been found to use glucose as a source of carbon than any other sugar (124) and according to present knowledge glucose appears to play a central role in metabolism, and hence should be a good basic compound for comparative purposes.

Nitrogen source.

Growth of O. graminis and F. annosus in media containing organic sources of nitrogen was very much superior to that in media containing nitrate or ammonium. Asparagine was chosen as nitrogen source and was used at 2.36 grams per litre of medium (i.e. 0.5 gram nitrogen). Asparagine has been found to be a satisfactory nitrogen source for many fungi (97, 124).

Major mineral sources.

In addition to oxygen, hydrogen, carbon and nitrogen, other elements required by fungi in appreciable amounts are potassium, magnesium, phosphorus and sulphur. These four elements were supplied as the salts KH_2PO_4 (1 gram per litre), $MgSO_4.7H_2O$ (0.5 gram per litre). These have been found to be satisfactory by numerous workers. References are given by Steinberg (188, 191).

Minor mineral sources.

The trace element nutrition of fungi has been the subject of considerable study by Steinberg and others, e.g. Steinberg (187) and has been reviewed by Perlman (156). Requirements have been demonstrated for iron, zinc, copper, manganese, molybdenum, calcium, gallium, scandium, vanadium, and cobalt (124). These requirements only become apparent where carefully purified media are used and in most ordinary circumstances are supplied as impurities in water and other constituents of the medium. Consequently, in this investigation, only three minor elements were added in the basic medium, iron, zinc and manganese, these having been reported probably to be required in greatest quantity. They were supplied as $Fe_2(SO_4)_3$, $ZnSO_4.7H_2O$, and $MnSO_4.4H_2O$, to give per litre of medium Fe ion

0.2 milligrams, Zn ion 0.2 milligrams, and Mn ion 0.1 milligrams.

These salts were made up in a stock solution such that 2 ml. contained the amounts indicated and was added to each litre of medium.

Vitamin supplement.

O. graminis and *F. annosus* both grew satisfactorily in synthetic media only when vitamins were present. The vitamins added to the basic medium had all been demonstrated to be essential for certain fungi; these, together with the concentrations used, were as follows:

Thiamine hydrochloride	100 micrograms per litre
Pyridoxine hydrochloride	100 micrograms per litre
Calcium d-pantothenate	100 micrograms per litre
Biotin	10 micrograms per litre
i-Inositol	5 milligrams per litre
Niacinamide	100 micrograms per litre
p-Aminobenzoic acid	100 micrograms per litre

Stock solutions (in 20% ethyl alcohol) of each of these substances were made up so as to contain the amount indicated above in 1 ml., simplifying addition of such small amounts of material to the medium. The solutions were stored at 0°C.

The complete basic medium had the composition:

D-glucose	12.51 grams
L-asparagine	2.36 grams
KH_2PO_4	1.00 grams
$MgSO_4.7H_2O$	0.50 grams
Fe ion	0.20 milligrams

Zn ion	0.20 milligrams
Mn ion	0.10 milligrams
Thiamine hydrochloride	100 micrograms
Pyridoxine hydrochloride	100 micrograms
Calcium d-pantothenate	100 micrograms
Biotin	10 micrograms
i-Inositol	5 milligrams
Niacinamide	100 micrograms
p-Aminobenzoic acid	100 micrograms
Agar (in solid media)	17.5 grams

Distilled water to make 1 litre.

The pH was adjusted to 5.5 with $\frac{N}{10}$ HCI or $\frac{N}{10}$ KOH before making up to the final volume.

GENERAL OBSERVATIONS ON GROWTH RATES OF *TRICHODERMA VIRIDE*, *TRICHOCLADIUM ASPERUM*, *OPHIOBOLUS GRAMINIS* AND *FOMES ANNOSUS*.

Garrett (75) observed that a high growth rate is typical of the common soil saprophytes, other than the lignin decomposers, this being a major factor in the rapid and successful colonization of such ephemeral substrates as the simpler sugars by these fungi. To determine how well this generalization applied to the four fungi under investigation a simple comparison of their growth rates was made.

MATERIALS AND METHODS.

A medium of the following composition was used for preparation of

the inoculum and comparison of the fungi:

Malt extract	5 grams
Yeast extract	5 grams
Dextrose	15 grams
Agar	17 grams

Distilled water to make 1 litre.

A complex medium of this type would be expected to provide optimum conditions of nutrition for the growth of all fungi.

Petri dishes containing 15 ml. of this medium were inoculated with plugs 5 m.m. in diameter cut from cultures of the four fungi growing at room temperature on medium prepared in the same way. Three Petri dishes were inoculated with each fungus and incubation was carried out at room temperature.

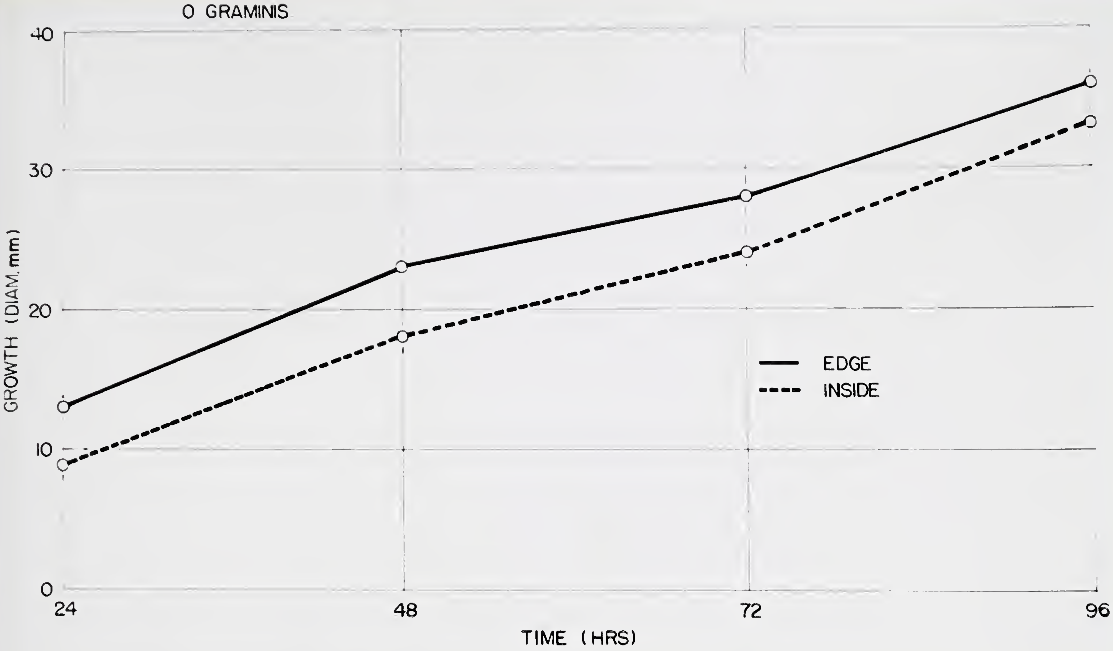
Measurements of the diameter in millimetres of the colonies were made each day or more frequently.

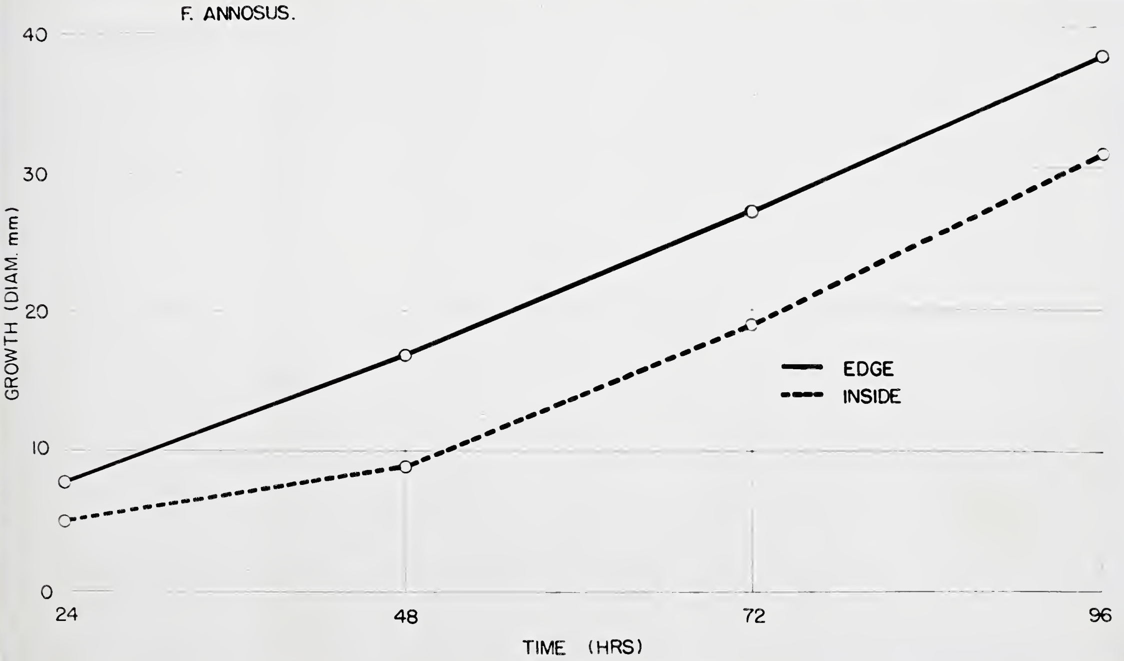
RESULTS.

Effect of the source of the inoculum on subsequent growth.

Observation of variation in the growth of agar cultures of *F. annosus* inoculated with pieces of mycelium taken at random from the surface of a stock culture suggested that the position from which the inoculum is taken from a culture may influence the subsequent behaviour of the fungus. The importance of uniform inoculum for experimental work was obvious and so 5 m.m. plugs were cut from the edge and from the adjacent position along a radius of cultures of both *F. annosus* and *O. graminis* and their growth rates compared. These are illustrated graphically in Figure 2. The

FIGURE 2. The effect of the source of the inoculum of Ophiobolus graminis and Fomes annosus on subsequent growth. Growth was measured as diameter of the fungus colony in millimetres.


O GRAMINIS
GROWTH (DIAM. mm)
40
30
20
10
0
24
48
72
96
TIME (HRS)
EDGE
INSIDE


F. ANNOSUS.
GROWTH (DIAM. mm)
40
30
20
10
0
24
48
72
96
TIME (HRS)
EDGE
INSIDE

growth rates are seen to be the same for the inoculum from the two sources, but initially there is a lag in growth rate for the plugs from the inside. This is most noticeable for F. annosus.

It is at once apparent that where growth rates are to be compared the effect of inoculum source may be of little significance, but where total growth is to be compared as in the liquid culture experiments, its importance might be considerable.

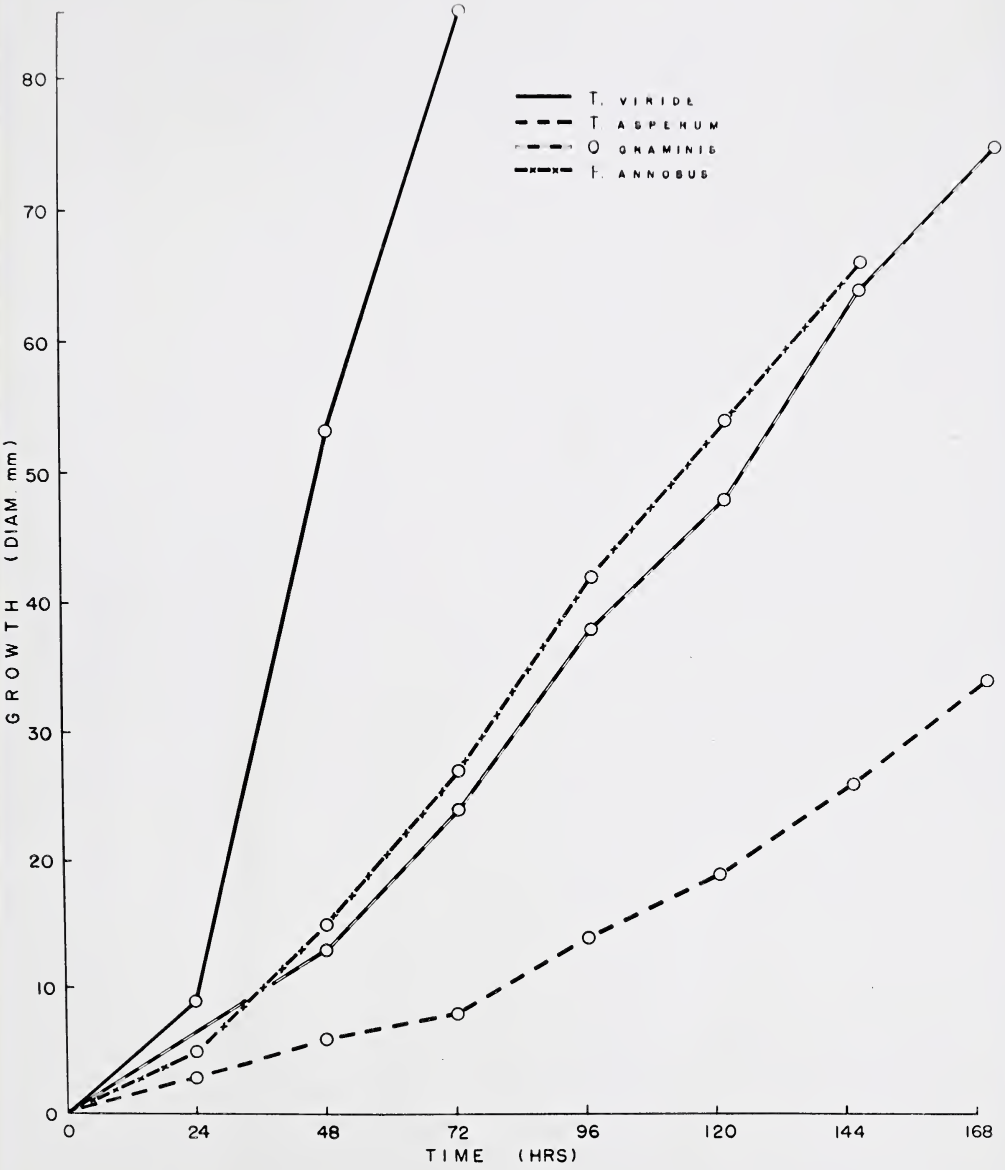
In all subsequent experiments where inoculum was obtained from cultures on agar, plugs were cut from the periphery or, if this was not possible, from points equally distant from the centre of the culture.

Growth rates of Trichoderma viride, Trichocladium asperum, Ophiobolus graminis and Fomes annosus.

The data for growth rates are given in Table 7 and graphically in Figure 3. Each value represents the average from three cultures.

Most conspicuous is the very high growth rate of T. viride as measured by this method. That such a high growth rate is to be expected from the observed behaviour of this fungus in the soil will be discussed later. By contrast, the second saprophyte has the slowest growth rate of the four fungi and is, therefore, not typical of the group according to Garrett's view (75). The growth rates of F. annosus and O. graminis were similar and about twice that of T. asperum. The growth of F. annosus was somewhat greater in this experiment than in many others where it was frequently observed to be somewhat slower than that of O. graminis, but the fact that high growth rate is not essential for saprophytic success and that the growth of parasitic fungi need not be slower than saprophytes are the main points to be drawn from these

FIGURE 3. Growth rates of _Trichoderma viride_, _Trichocladium asperum_, _Ophiobolus graminis_ and _Fomes annosus_ on a solidified organic medium. Growth was measured as the diameter of the fungus colony in millimetres.


T. VIRIDE
T. ASPERUM
O. GRAMINIS
F. ANNOSUS
GROWTH (DIAM. mm)
0
10
20
30
40
50
60
70
80
0
24
48
72
96
120
144
168
TIME (HRS)

observations.

TABLE 7. COMPARATIVE GROWTH OF *TRICHODERMA VIRIDE*, *TRICHOCLADIUM ASPERUM*, *OPHIOBOLUS GRAMINIS* AND *FOMES ANNOSUS* ON A SOLIDIFIED ORGANIC MEDIUM.

	GROWTH AS DIAMETER OF FUNGUS COLONY IN MILLIMETRES						
	TIME (hrs.)						
	24	48	72	96	120	144	168
TRICHODERMA VIRIDE	9.3	52.0	85.0	–[1]			
TRICHOCLADIUM ASPERUM	3.3	6.0	8.3	14.3	19.3	26.3	33.6
OPHIOBOLUS GRAMINIS		13.3	23.6	37.6	48.0	63.5	76.5
FOMES ANNOSUS	4.6	15.0	27.0	42.0	53.6	66.3	-

[1] Plate completely filled.

EFFECT OF TEMPERATURE ON GROWTH OF *TRICHODERMA VIRIDE*, *TRICHOCLADIUM ASPERUM*, *OPHIOBOLUS GRAMINIS* AND *FOMES ANNOSUS*.

The cardinal points of temperature of fungi are usually in the region of 0-5°C., 20-30°C., and 30-35°C. (97), but the variations between different fungi and even different strains of the same fungus may be quite marked. Although the majority of experiments reported in these investigations were carried out at room temperature, it was of interest to compare the effect of temperature on growth, a) to ascertain if room

temperature would be satisfactory for the growth of all fungi, and b) to uncover any differences between the fungi in response to temperature that might have significance in their natural habitats.

MATERIALS AND METHODS.

The procedure used here was the same as that used in the previous experiment, except that cultures were incubated at 10°C., 15°C., 20°C., 25°C., 30°C., 35°C. and 40°C. Three cultures of each fungus were grown at each of these temperatures. The incubators used were fitted either with a refrigeration unit (10°C., 15°C. and 20°C.), or a heating unit (25°C., 30°C., 35°C. and 40°C.), and except for the 25°C. unit temperature control was within 1°C. Somewhat larger fluctuations occasionally occurred in the 25°C. cabinet due to variations in the room temperature. No control of humidity was available and so in addition to differences in temperature between the incubators there would also be large differences in humidity. The effect of humidity on the growth of the fungi was not determined independently, although it should be stated that differences in humidity have been shown to alter the temperature optima of *Aspergillus niger* (13) and of *Penicillium chrysogenum* (90).

RESULTS.

The results are given in Table 8 and in Figures 4 and 5. In Figure 4 the growth rates are illustrated while in Figure 5 total growth after 120 hours is shown.

The data indicate that considerable differences existed in the temperature response of the isolates of the fungi studied. For *T. viride*

the optimum temperature was probably between 25°C. and 30°C., higher than that of the other three fungi. T. viride was the only fungus to make any growth at all at 35°C., although here the growth was soon arrested. Growth of T. viride was much retarded at the lower temperatures, even at 15°C. there was a lag of 2½ days.

T. asperum had a much lower optimum temperature (20-25°C) than T. viride and 30°C. was inhibitory. Growth at 10 and 15°C. was, however, comparatively good.

O. graminis, like T. asperum, was inhibited by a temperature of 30°C. and its optimum temperature was also between 20 and 25°C. The ability of this fungus to grow at 10 and 15°C. was quite marked.

The considerable lag before onset of growth for F. annosus was somewhat unusual. There was, however, a quite pronounced optimum at 25°C., with no growth at 35°C and only a trace of growth at 15°C.

References in the literature to the influence of temperature on the growth of any of the fungi considered here are few. Rishbeth (165) observed the growth rates of F. annosus and T. viride in Petri dishes at 10°C., 5°C., and 0°C. At 10°C.the growth of T. viride was greater than that of F. annosus; at 5°C. the growth rates were similar, while at 0°C. F. annosus grew faster than T. viride, although growth was very slight. No indication was given of the length of time over which these observations were made, but comparison with the results obtained in the present study suggests that the response at least of F. annosus to temperature was markedly different. This could be a characteristic difference between the two isolates or could be due to adaptation if Rishbeth's cultures were incubated for a long period.

TABLE 8. EFFECT OF TEMPERATURE ON THE GROWTH OF TRICHODERMA VIRIDE, TRICHOCLADIUM ASPERUM, OPHIOBOLUS GRAMINIS AND FOMES ANNOSUS.

a) TRICHODERMA VIRIDE.

TEMPERATURE °C	GROWTH AS DIAMETER OF FUNGUS COLONY IN MILLIMETRES. TIME (hrs.)									
	24	41	48	60	72	84	96	108	120	132
10	0	0	0	0	0	5.0	7.4	11.0	13.6	15.3
15	0	0	0	0	7.0	12.3	20.0	28.3	35.6	46.3
20	0	16.6	22.3	34.6	47.3	60.3	75.6	–[1]		
25	12.0	45.0	60.0	85.0	–					
30	16.3	53.6	69.6	–						
35	8.3	16.6	16.6	16.6	16.6	16.6	16.6	16.6	16.6	16.6
40	0	0	0	0	0	0	0	0	0	0

---

[1] Plate completely filled.

TABLE 8 (CONTINUED).

b) TRICHOCLADIUM ASPERUM.

TEMPERATURE °C	GROWTH AS DIAMETER OF FUNGUS COLONY IN MILLIMETRES. TIME (hrs.) 24	41	48	60	72	84	96	108	120	132
10	0	4.0	4.6	6.0	7.0	9.0	0.6	10.3	11.0	12.3
15	0	4.0	4.8	6.6	8.0	9.6	11.3	13.0	15.0	17.0
20	4.0	7.0	8.3	10.0	12.6	16.0	19.0	21.6	24.3	27.0
25	5.0	8.0	9.3	10.3	11.3	13.6	16.0	18.0	21.6	22.6
30	4.3	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6
35	0	0	0	0	0	0	0	0	0	0

TABLE 8 (CONTINUED).

c) *OPHIOBOLUS GRAMINIS*.

TEMPERATURE GROWTH AS DIAMETER OF FUNGUS COLONY IN MILLIMETRES.

°C	TIME (hrs.)									
	24	41	48	60	72	84	96	108	120	132
10	0	4.6	6.0	8.0	10.0	11.6	13.3	15.3	19.0	20.0
15	0	5.4	6.0	9.0	11.0	15.3	17.0	21.0	25.0	28.0
20	4.0	9.6	12.0	16.3	21.0	28.0	32.0	38.6	44.3	49.3
25	6.3	11.3	12.3	17.0	18.6	24.0	29.3	35.0	41.6	47.6
30	4.3	6.3	7.3	9.0	9.0	9.0	9.0	9;0	9.0	9.0
35	0	0	0	0	0	0	0	0	0	0

TABLE 8 (CONTINUED).

d) FOMES ANNOSUS.

TEMPERATURE	GROWTH AS DIAMETER OF FUNGUS COLONY IN MILLIMETRES.									
°C	TIME (hrs.)									
	24	41	48	60	72	84	96	108	120	132
15	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	1.0	4.3	6.3	9.3	12.0	14.6
25	0	0	0	2.0	4.6	9.0	12.6	19.0	24.6	30.0
30	0	0	0	2.0	3.0	4.3	6.3	8.3	10.3	11.6
35	0	0	0	0	0	0	0	0	0	0

FIGURE 4. Effect of temperature on the growth rates of *Trichoderma viride*, *Trichocladium asperum*, *Ophiobolus graminis* and *Fomes annosus* on a solidified organic medium. Growth was measured as the diameter of the fungus colony in millimetres.

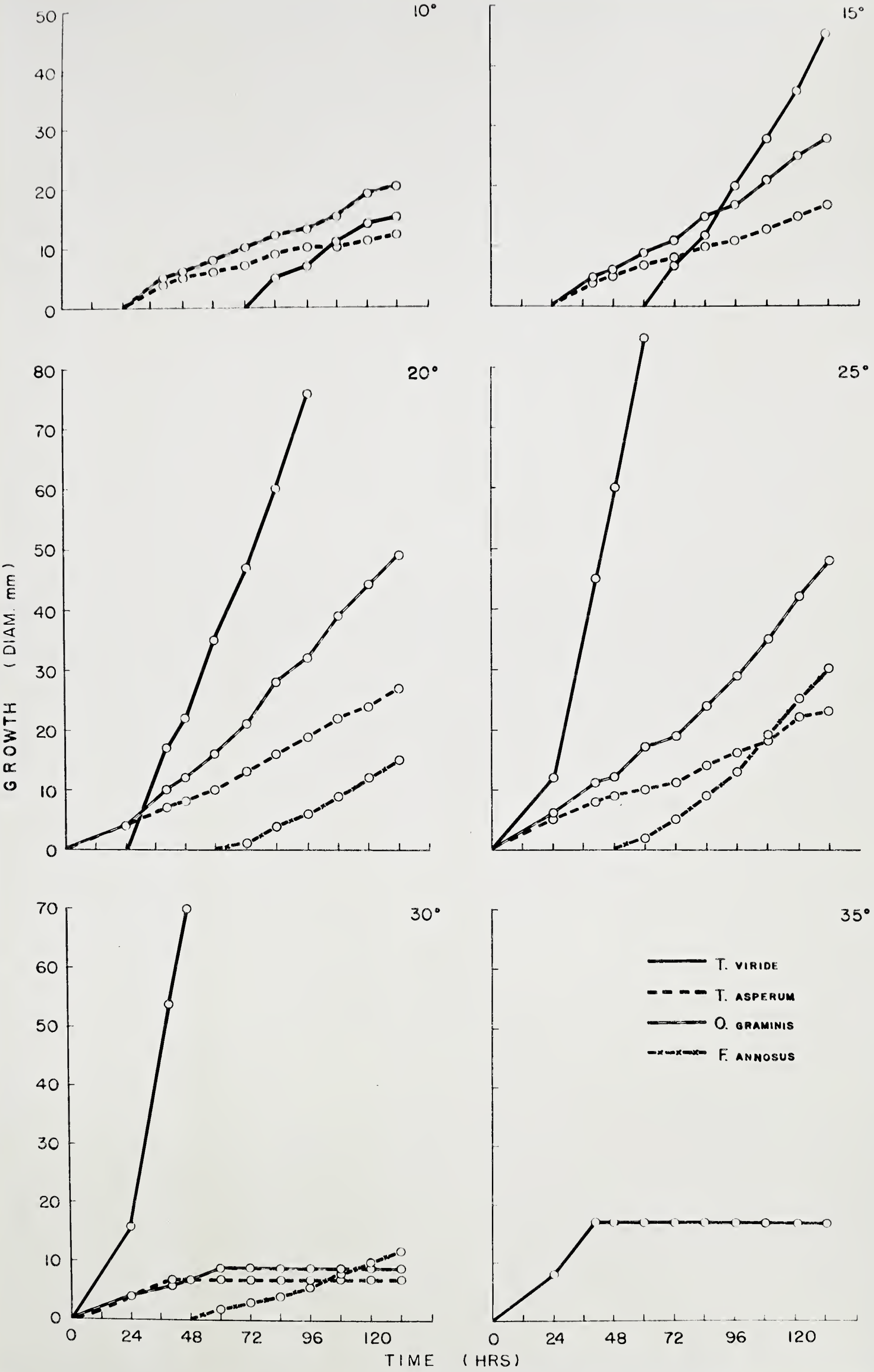
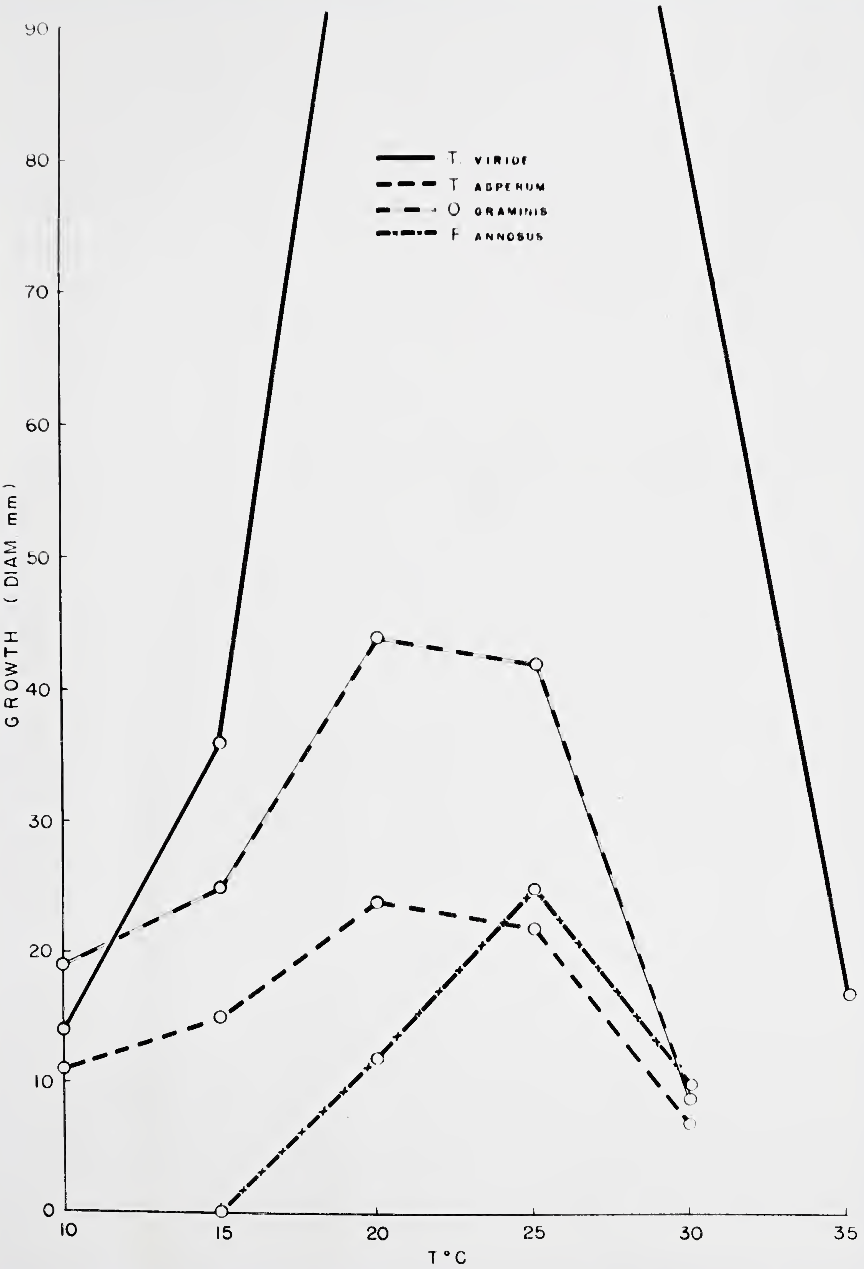

10°
15°
20°
25°
30°
35°
50
40
30
20
10
0
80
70
60
50
40
30
20
10
0
70
60
50
40
30
20
10
0
GROWTH (DIAM. mm)
0
24
48
72
96
120
TIME (HRS)
T. VIRIDE
T. ASPERUM
O. GRAMINIS
F. ANNOSUS

FIGURE 5. Effect of temperature on the total growth of *Trichoderma viride*, *Trichocladium asperum*, *Ophiobolus graminis* and *Fomes annosus*. Growth was measured as the diameter of the fungus colony in millimetres after 120 hours.


T. VIRIDE
T. ASPERUM
O. GRAMINIS
F. ANNOSUS
GROWTH (DIAM mm)
90
80
70
60
50
40
30
20
10
0
10
15
20
25
30
35
T °C

Evidence for adaptation to temperature change was illustrated in the present investigation by both F. annosus and T. viride, at 15°C. where growth commenced after a lag. Other investigators have noted that the apparent temperature response of fungi is influenced considerably by the temperature at which a culture has been previously grown (Fawcett (57)).

The results obtained here for T. viride agree reasonably closely with those of Ludwig (128) for the same fungus except that he reported growth to occur at 40°C. and gave a somewhat higher optimum; growth at the lower temperatures was similarly initially poor but showed good adaptation.

Cartwright and Findlay (41) report 23°C. to be the optimum temperature for F. annosus with growth occurring chiefly between 20°C. and 27°C. Humphrey and Siggers (105) also list F. annosus as having an optimum temperature between 20°C. and 24°C. The results reported above tally fairly closely with those of Cartwright and Findlay, and Humphrey and Siggers.

No references to the effect of temperature on the growth of T. asperum have been found in the literature.

The effect of temperature on the growth of O. graminis on potato-dextrose agar was studied by Davis (49). Three isolates were used and all showed similarity in having minimal, optimal and maximal temperatures of approximately 4°C., 23-24°C. and 33°C. respectively. A similar study was carried out by Davies (47) who used four different isolates of O. graminis; differences between the isolates were demonstrated but the optimum was probably within the range 20-25°C. In both these reports growth was recorded as a total and hence differences between isolates could have been due to differences in the inoculum used, and final growth

rates may have been more similar than is suggested by the results. However, it may be concluded that the temperature response found for O. graminis in the present investigation was comparable to that observed by other authors.

Other experiments recorded here were carried out at room temperature. This approximated within reasonable limits the optima for the fungi studied, and lack of controlled temperatures, although obviously undesirable, is not considered too severe a handicap in drawing conclusions.

From the point of view of the general problem of determining differences between the fungi used in this study the temperature responses, particularly of O. graminis and T. viride, illustrate that this factor alone could be a major one in determining saprophytic success in the soil.

EFFECT OF pH OF THE MEDIUM ON GROWTH OF TRICHODERMA VIRIDE, TRICHOCLADIUM ASPERUM, OPHIOBOLUS GRAMINIS AND FOMES ANNOSUS.

The importance of the pH of the medium on growth is illustrated, as pointed out by Wolf and Wolf (238) for example, in the simple procedure of acidification of media intended for the isolation of fungi from mixed cultures of bacteria and fungi. Bacteria as a group tend to prefer a neutral reaction. Norkrans (148) observes that the effect of hydrogen-ion concentration depends largely on the kinds and concentrations of the other ions present in the medium, and that pH relations found to apply under one set of cultural conditions may be expected to apply strictly only under those conditions. Hydrogen-ion concentration has long been known to be a

critical factor for enzyme action and hence optimal pH for growth on a particular substrate may be expected to be that pH coinciding most closely with the pH optima of the enzymes required to metabolize that substrate. In this connection the work of Naguib (142) is of interest. He showed that inhibition of growth of *Fusarium moniliforme* at pH 4.0 on a medium containing sucrose could be correlated with reduction in invertase activity, and of sugar uptake at this pH. Lilly and Barnett (124) also draw attention to the correlation which may exist between the effect of pH on growth and on enzyme activity. In addition, pH is known to be a factor influencing the absorption of ions from the medium (32, 252).

In spite of these observations it does appear to be true that many species have a characteristic pH range for growth. Experiments were, therefore, carried out to obtain information on the pH range of *T. viride*, *T. asperum*, *O. graminis* and *F. annosus*.

MATERIALS AND METHODS.

Liquid cultures were employed for these experiments due to the fact that acidified agar frequently fails to solidify, and growth was determined by the dry weight of mycelium produced.

A medium of the following composition was employed:

Asparagine	2.36 grams
Glucose	15.00 grams
$Mg.SO_4.7H_2O$	0.50 grams
Fe ion, Zn ion, Mn ion.	
Vitamin mixture	
Citrate-phosphate buffer	340 millilitres
Distilled water to make 1 litre	

This is essentially the basic medium described earlier. Asparagine was used as nitrogen source here a) because it was known to support better growth of the parasitic fungi than inorganic nitrogen sources, and b) because its utilization is likely to result in less change in the pH of the medium than is true for inorganic nitrogenous compounds (Norkrans (148)).

In order to reduce pH changes during growth to a minimum it seemed advisable to use a buffer. Use of a buffer does have disadvantages in that it introduces ions additional to those required for nutrition into the culture medium, sometimes in rather high concentration and frequently in different proportions at different pH levels. This means that cultural conditions at different hydrogen-ion concentrations may not be strictly comparable. There are few buffer systems which have a satisfactory range for use in nutritional experiments of the kind performed here. Norkrans (148) and Lindeberg (127) used a 0.04 M phosphate buffer comprised of various combinations of $\frac{1}{15}$M solutions of K_3PO_4, Na_2HPO_4, KH_2PO_4 with $\frac{1}{10}$N HCI in studies of the pH range of various basidiomycetes. Treschow (206) observed, however, that a phosphate concentration of 0.066 M prevented the growth of Psalliota bispora and one tenth this concentration retarded growth. Naguib (142) used succinic acid and Na_2HPO_4 to control the pH of the medium in his experiments.

In the experiments reported here various proportions of 0.1M citric acid and 0.2 M K_2HPO_4 were used to give solutions with pH values of 3.0, 4.0, 5.0, 6.0 and 7.0 as determined using a Beckman pH meter (model H2). These solutions were then incorporated in the final medium, making up one third of the total volume. The obvious disadvantage of the buffer used was that citrate may act as a nutrient and hence give erroneous

results for the more acid pH values. However, as mentioned by Steinberg (188), growth on organic acids as carbon source, including citric, is usually poor, and Foster (61) points out that in the commercial production of citric acid low pH favours high yields from carbohydrate, the carbohydrate being used preferentially as long as it is available in sufficient quantity.

200 ml. Erlenmeyer flasks containing 20 ml. of the adjusted medium were autoclaved for 8 minutes under standard conditions, the pH after autoclaving was determined and the values are given in Table 9. The flasks were inoculated with 5 m.m. plugs taken from the periphery of cultures of the fungi growing on the same medium adjusted to pH 5.5 but solidified with agar. The inoculum plug was supported on the bent tip of a glass rod as described previously. Four flasks for each pH were used for each fungus and the mycelium was harvested, dried and weighed after the period of growth indicated in Table 9.

RESULTS.

The dry weights of mycelium in m.m. obtained for the various pH values are given in Table 9 and are reproduced graphically in Figure 6.

Considerable variation occurred between flasks of the same treatment, which has resulted in a lack of statistical significance between some of the values obtained. However, in spite of this certain features stand out clearly.

Both saprophytes are able to make good growth under more acid conditions than the parasites. In fact, at pH 3.05 and pH 4.00 growth of *O. graminis* and *F. annosus* was only slight whereas that of *T. viride*

TABLE 9. EFFECT OF pH ON GROWTH OF *TRICHODERMA VIRIDE*, *TRICHOCLADIUM ASPERUM*, *OPHIOBOLUS GRAMINIS* AND *FOMES ANNOSUS*.

a) *TRICHODERMA VIRIDE*.

	pH[1]	MILLIGRAMS DRY MYCELIUM PER FLASK				TOTAL	MEAN
6 days growth	3.05	110.7	99.7	109.1	113.5	433.0	108.25
	4.00	127.0	124.5	102.5	112.0	467.0	116.75
	5.10	145.0	151.0	132.5	137.5	566.0	141.5
	6.20	114.5	136.5	131.0	132.5	514.5	128.6
	6.90	138.0	150.5	137.5	151.5	577.5	144.4

---

[1] pH after autoclaving.

ANALYSIS OF VARIANCE.

SOURCE OF VARIATION.	D.F.	M.S.	F.	F. at 1%	L.S.D. at 1%
Treatments	4	967.35	14.64	4.89	16.94
Error	15	66.07			
Total	19				

TABLE 9 (CONTINUED).

b) TRICHOCLADIUM ASPERUM.

	pH[1]	MILLIGRAMS DRY MYCELIUM PER FLASK				TOTAL	MEAN
17 days growth	3.05	86.5	96.0	91.5	55.0	257.0	64.3
	4.00	144.0	154.0	159.5	184.0	641.5	160.4
	5.10	114.0	125.0	139.0	130.5	508.5	127.1
	6.20	126.0	134.0	147.0	122.0	509.0	132.3
	6.90	170.5	149.5	113.5	117.0	550.0	137.6

[1] pH after autoclaving.

ANALYSIS OF VARIANCE.

SOURCE OF VARIATION.	D.F.	M.S.	F.	F. at 1%	L.S.D. at 1%
Treatments	4	5152.83	10.28	4.89	46.65
Error	15	501.31			
Total	19				

TABLE 9 (CONTINUED)

c) *OPHIOBOLUS GRAMINIS*.

	pH[1]	MILLIGRAMS DRY MYCELIUM PER FLASK				TOTAL	MEAN
17 days growth	3.05	43.0	34.5	52.5	29.0	159.0	39.8
	4.00	35.0	23.0	15.0	33.5	106.5	26.6
	5.10	121.0	139.0	171.0	133.5	564.5	141.1
	6.20	88.5	144.0	101.5	157.0	491.0	122.8
	6.90	137.5	159.0	111.5	157.5	565.5	141.4

[1] pH after autoclaving.

ANALYSIS OF VARIANCE.

SOURCE OF VARIATION.	D.F.	M.S.	F.	F. at 1%.	L.S.D. at 1%
Treatments	4	12773.64	28.73	4.89	43.94
Error	15	444.64			
Total	19				

TABLE 9 (CONTINUED).

d) FOMES ANNOSUS.

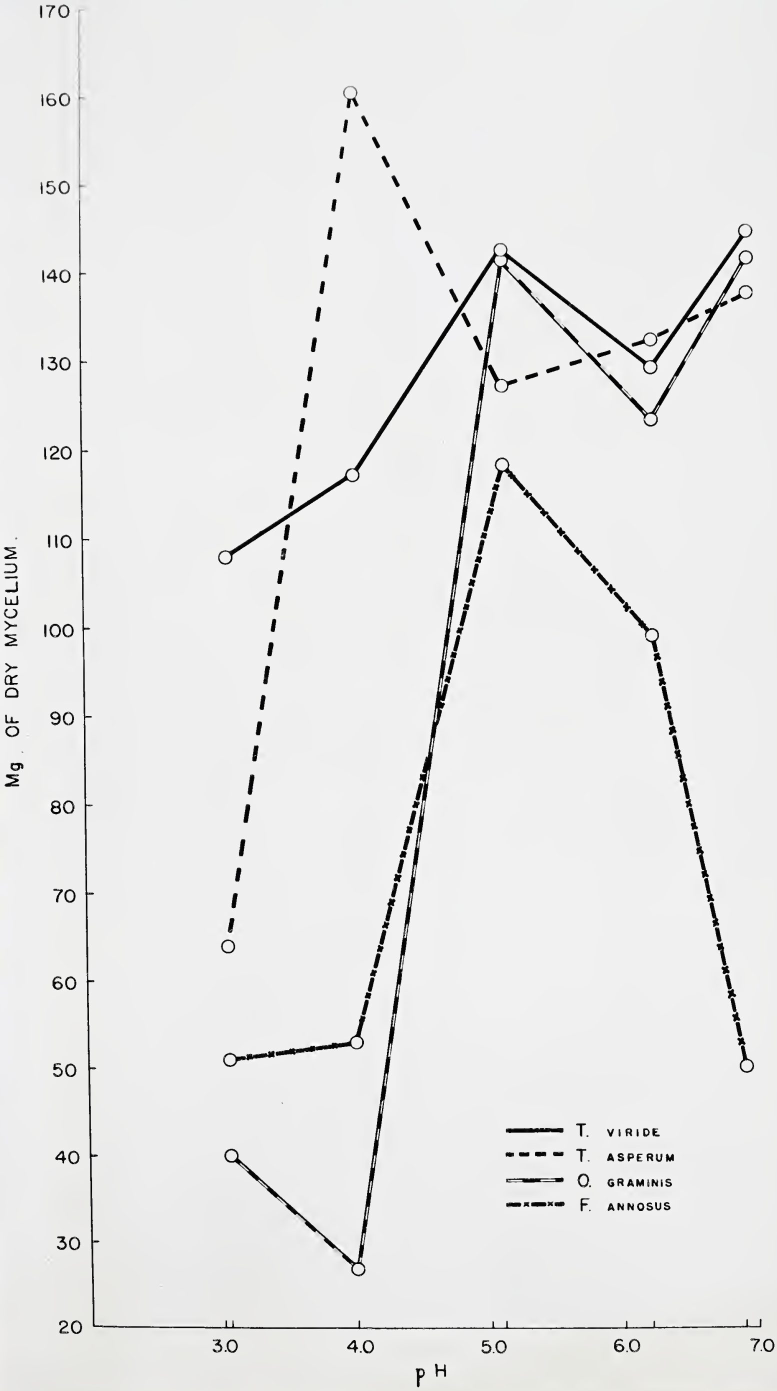
	pH[1]	MILLIGRAMS DRY MYCELIUM PER FLASK				TOTAL	MEAN
17 days growth	3.05	76.5	38.5	49.0	40.5	204.5	51.1
	4.00	61.0	48.5	57.0	44.5	211.0	52.8
	5.10	115.0	128.0	84.0	105.0	432.0	108.0
	6.20	82.0	110.5	93.5	110.0	396.0	99.0
	6.90	57.0	46.0	44.5	53.5	201.0	50.3

[1] pH after autoclaving.

ANALYSIS OF VARIANCE.

SOURCE OF VARIATION.	D.F.	M.S.	F.	F. at 1%	L.S.D. at 1%
Treatments	4	3304.15	17.87	4.89	28.50
Error	15	187.11			
Total	19				

FIGURE 6. Effect of pH of the medium on the growth of *Trichoderma viride*, *Trichocladium asperum*, *Ophiobolus graminis* and *Fomes annosus*. Growth was recorded as the weight of dry mycelium in milligrams.


Mg. OF DRY MYCELIUM.
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
3.0
4.0
5.0
6.0
7.0
p H
T. VIRIDE
T. ASPERUM
O. GRAMINIS
F. ANNOSUS

was not much less than that at higher pH values. That of T. asperum, although poor at the lower pH, was significantly higher at pH 4.00 and here growth was as great as at any other pH value. It was of interest to note, however, that final growth of O. graminis at pH 4,00, 5.10, 6.20 and 6.90 was as great as that of either T. viride or T. asperum. For these three fungi the higher pH limits of growth were not determined, and there did not appear to be any clear-cut optimum. For F. annosus the pH range for growth was apparently much narrower, lying chiefly between pH 4.00 and 6.90, with an optimum value probably between pH 5.10 and 6.20. Davis (49) found the pH optima of three strains of O. graminis to vary between 5.6 and 7.6 and obtained good growth up to pH 9.0.

There are several references in the literature to the pH range of F. annosus. Weis and Nielson (228) found pH 4.0 - 4.5 to be the most favourable range, but mention the results of Lagerberg and Melin who give pH 6.0 as the optimum. Robak (168) found the growth range of F. annosus on sawdust adjusted to various pH values with hydrochloric acid to lie between 4.0 and 6.3. Tilford (204) found the optimum pH for F. annosus on malt agar to be approximately 5.0. Treschow (205) found the optimum pH to be 4.0 - 4.4 on 3% malt extract and obtained no growth below pH 3.0 or above pH 6.0. Thus there appears to be some variation in the values obtained by different authors, which may be due to differences in strains. The observations of Robak and of Tilford agree reasonably closely with those of the writer.

In conclusion it may be stated that high acidity was distinctly more disadvantageous to the parasites than to the saprophytes under the conditions of these experiments, but under more alkaline conditions, while

F. annosus was inhibited, O. graminis made as good growth as either T. viride or T. asperum.

On the basis of the observations made here media in subsequent experiments were adjusted to an initial pH value of 5.5.

EFFECT OF NITROGEN SOURCE ON GROWTH OF TRICHODERMA VIRIDE, TRICHOCLADIUM ASPERUM, OPHIOBOLUS GRAMINIS AND FOMES ANNOSUS.

Robbins (169) divided fungi into four groups according to their ability to utilize different sources of nitrogen as follows: (1) able to use atmospheric, nitrate, ammonium and organic nitrogen; (2) able to use nitrate, ammonium and organic nitrogen only; (3) able to use ammonium and organic nitrogen only; (4) able to use organic nitrogen only. The concensus of opinion at the present time is that utilization of atmospheric nitrogen by fungi has not been satisfactorily demonstrated, (238), but the last three groups seem to hold satisfactorily, although exceptions have been reported. Thus Brian et al. (26) reported that Metarrhizium glutinosum was able to grow on nitrate nitrogen but not on ammonium nitrogen, and Newton (143) observed better growth of Alternaria solani on nitrate than on ammonium nitrogen.

Some attempt has been made by various investigators to give a physiological explanation of the differences in nitrogen requirements found among fungi. The pathway of nitrate nitrogen utilization in Torula utilis has been studied by Virtanen and Csaky (211) and Virtanen et al. (212). Nitrate was first reduced to hydroxylamine and this

substance then combined with alpha-keto acids; the subsequent pathway was identical to that of ammonium nitrogen, a sequence that has long been considered to occur in higher plants (202). Thus a fungus unable to use nitrate nitrogen is probably deficient for a mechanism necessary to carry out one of these initial steps, if it can use ammonium nitrogen.

Utilization of an amino acid may be by direct incorporation into protein or by deamination giving ammonia. However, where growth occurs on one amino acid, synthesis of the other amino acid components of protein must involve transamination, and hence ammonium should be the preferred form of nitrogen. Two explanations have been put forward to account for the fact that it frequently is not. One is that the presence or utilization of ammonium may cause unfavourable conditions of pH in the medium (93), and the second is that a lack of suitable ammonium acceptors prevents conversion of ammonia to amino acids. Evidence in support of the second explanation has been obtained by several workers who have demonstrated that addition of various organic acids to culture media containing ammonium as nitrogen source results in an increase in the utilization of ammonium nitrogen (26, 93, 118, 122, 149, 161). Thus ability to grow only on an organic nitrogen source probably represents a deficiency in the production of the four carbon dicarboxylic acids.

It is apparent, therefore, that ability to utilize nitrogen on the part of a fungus indicates more adequate enzymatic equipment than is possessed by those fungi able to grow only on ammonium or organic nitrogen, and similarly those which grow only on organic nitrogen may be considered inferior in this respect to those which are able to grow on ammonium nitrogen.

A consideration of the utilization of the various forms of nitrogen by T. viride, T. asperum, O. graminis and F. annosus was, therefore of fundamental interest.

Both O. graminis and F. annosus were known to require vitamins; growth was compared on four different nitrogen sources with and without added vitamins.

MATERIALS AND METHODS.

The medium was as follows:

Glucose	12.51 grams
KH_2PO_4	1.00 grams
$MgSO_4.7H_2O$	0.50 grams
Fe ion, Zn ion, Mn ion.	
Agar	17.00 grams

Distilled water to make 1 litre.

This was supplemented with the following 8 combinations of nitrogen source and vitamin supplement. The vitamins used were indicated earlier during the discussion of the basic medium.

	No vitamins	Vitamins added
Potassium nitrate (3.6 grams)	1	5
Ammonium sulphate (2.34 grams)	2	6
L-Asparagine (2.36 grams)	3	7
Yeast hydrolysate (4.54 grams)	4	8

The nitrogen sources were added at the rate of 0.5 grams per litre, the weight of yeast hydrolysate being based upon the figure of 11.2% nitrogen given by the manufacturer.

Petri dishes containing 15 ml. of medium were inoculated in the usual way with plugs from cultures growing on a medium corresponding to combination 7 above. Three dishes of each medium were inoculated with each fungus and measurements were made of growth in millimetres.

RESULTS.

The data are given in Table 10.

The most conspicuous feature is that only T. viride and F. annosus made any growth on media containing ammonium sulphate, although White (232) demonstrated that ammonium nitrate could be used as a nitrogen source for O. graminis. The probable explanation is that the utilization of the ammonium ion by the fungi resulted in acid conditions in the medium unfavourable to the growth of O. graminis and T. asperum. Growth of both T. viride and F. annosus on ammonium sulphate was much reduced compared with that on the other sources of nitrogen.

Both T. viride and T. asperum made as good growth on potassium nitrate as on asparagine and although growth of T. viride was better on yeast hydrolysate than on the other sources of nitrogen, that of T. asperum was much the same. However, growth on asparagine and yeast hydrolysate was more dense than on the inorganic nitrogen. There was no indication of any vitamin requirement by either of these fungi. Both would fall into Group 2 of Robbin's classification.

Neither O. graminis nor F. annosus grew satisfactorily on any single nitrogen source (except yeast hydrolysate) without the addition of the vitamin supplement. Given a supply of vitamins both made better growth on asparagine than on inorganic nitrogen. Although the figures in Table

TABLE 10. EFFECT OF NITROGEN SOURCE AND VITAMINS ON THE GROWTH OF *TRICHODERMA VIRIDE*, *TRICHOCLADIUM ASPERUM*, *OPHIOBOLUS GRAMINIS* AND *FOMES ANNOSUS* IN CULTURE.

a) *TRICHODERMA VIRIDE*.	GROWTH AS DIAMETER OF FUNGUS COLONY IN MILLIMETRES.				
	TIME (hrs.)				
	24	36	48	60	72
Potassium nitrate	8.3	23.0	40.0	58.6	77.0
Potassium nitrate plus vitamins	8.0	21.3	40.6	59.0	78.3
Ammonium sulphate	5.0	14.0	30.6	48.3	66.6
Ammonium sulphate plus vitamins	3.6	10.6	28.3	42.3	62.0
Asparagine	9.6	23.3	40.6	58.6	77.3
Asparagine plus vitamins	9.3	22.3	41.3	59.3	78.3
Yeast hydrolysate	10.6	28.3	49.0	70.6	-[1]
Yeast hydrolysate plus vitamins	12.0	30.3	50.3	72.3	-

[1] Plate completely filled.

TABLE 10 (CONTINUED).

b) *TRICHOCLADIUM ASPERUM.*[2] GROWTH AS DIAMETER OF FUNGUS COLONY IN MILLIMETRES.

	TIME (hrs.)								
	24	36	48	60	72	84	108	132	156
Potassium nitrate	4.3	6.6	10.3	13.3	14.6	18.0	23.0	29.0	29.6
Potassium nitrate plus vitamins	4.6	8.0	11.0	12.6	15.0	17.6	23.3	29.3	30.3
Asparagine	4.0	6.6	8.3	10.6	12.3	15.0	19.0	24.0	26.0
Asparagine plus vitamins	4.0	6.6	8.6	10.6	13.3	15.3	19.3	24.6	26.0
Yeast hydrolysate	5.6	7.0	10.6	13.3	16.3	19.3	25.0	30.6	31.3
Yeast hydrolysate plus vitamins	6.3	7.3	10.6	14.0	16.0	19.0	26.3	30.6	32.6

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[2] Negligible growth occurred on ammonium sulphate.

TABLE 10 (CONTINUED).

c) OPHIOBOLUS GRAMINIS.	GROWTH AS DIAMETER OF FUNGUS COLONY IN MILLIMETRES.						
	TIME (hrs.)						
	48	60	72	84	108	132	156
Potassium nitrate	4.0	6.0	7.0	8.6	9.6	10.0	10.0
Potassium nitrate plus vitamins	4.0	7.3	9.3	14.0	19.6	31.3	40.6
Asparagine	5.3	9.0	0.6	11.3	11.3	12.0	12.0
Asparagine plus vitamins	6.0	10.3	14.6	19.3	31.3	46.6	58.0
Yeast hydrolysate	7.3	12.6	15.6	22.0	32.3	46.3	57.0
Yeast hydrolysate plus vitamins	7.6	12.0	14.6	22.0	31.3	44.6	56.3

TABLE 10 (CONTINUED).

d) FOMES ANNOSUS.	GROWTH AS DIAMTER OF FUNGUS COLONY IN MILLIMETRES.							
	TIME (hrs.)							
	48	60	72	84	108	132	156	180
Potassium nitrate	0	0	0	0	0	7.0		9.6[3]
Potassium nitrate plus vitamins	0	0	0	9.3	16.0	27.6	33.3	42.6[3]
Ammonium sulphate	0	0	0	0	0	4.0	4.0	5.3
Ammonium sulphate plus vitamins [4]	0	0	0	6.0	11.0	18.0	20.0	26.0
Asparagine	0	0	0	4.3	8.6	9.0	12.3	15.0
Asparagine plus vitamins	0	5.3	7.6	12.6	18.3	31.0	37.3	45.3
Yeast hydrolysate	3.6	5.3	10.6	16.0	27.3	42.3	51.0	59.0
Yeast hydrolysate plus vitamins	6.6	10.0	14.6	23.0	36.3	50.3	60.3	69.6

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[3] Starvation type growth.

[4] Average of two plates only.

10 indicate growth of F. annosus on potassium nitrate plus vitamins to be almost as great as that on asparagine plus vitamins, growth on the former was strictly a starvation type of growth and was not comparable to that on asparagine. It was of interest to note that even starvation type growth was much reduced in the absence of vitamins. As in the case of T. viride growth of F. annosus on yeast hydrolysate was superior to that on asparagine plus vitamins. Stimulation of this kind has been observed by many investigators and is considered to be due to either unknown growth factors or to the availability of already synthesized material that can be used directly in growth. O. graminis grew well on potassium nitrate when vitamins were present in the medium, and under these conditions nitrate may be considered a satisfactory nitrogen source for this fungus. Growth on asparagine was considerably better than that on potassium nitrate, while that on yeast hydrolysate was much more dense than on asparagine. O. graminis would probably be placed in Group 2 of Robbins classification while F. annosus would appear to be more typical of Group 3.

The observations reported here confirm the results of other investigators. Thus Hacskaylo et al. (93) found potassium nitrate and asparagine to be satisfactory nitrogen sources for T. viride and ammonium sulphate somewhat less favourable. According to Steinberg (191) utilization of nitrate nitrogen by T. viride was also reported by Blumer (11).

Jennison et al. (110) indicated that nitrate nitrogen was unsatisfactory for F. annosus and for several related species. Hacskaylo et al. (93) reported that F. subrosea grew poorly on potassium nitrate and that all Basidiomycetes tested, with one exception, made slight growth on this

nitrogen source.

O. graminis was first reported as requiring complex organic nitrogen for growth (59), but it was subsequently demonstrated by Garrett (71) that growth would occur on nitrate as nitrogen source. White (232) demonstrated that growth of O. graminis would occur on nitrate provided certain vitamins were also present in the medium. This observation was also confirmed by Gilpatrick (79).

Thus, in conclusion, it may be stated that the saprophytes studied in this investigation were able to make somewhat better use of the simpler forms of nitrogen than were the parasites. The vitamin requirements of the latter are considered in greater detail below.

THE VITAMIN REQUIREMENTS OF OPHIOBOLUS GRAMINIS AND FOMES ANNOSUS.

The data given in the previous section of this report indicate that satisfactory growth of O. graminis and F. annosus only occurred when a mixture of vitamins was added to the medium, and that T. viride and T. asperum grew satisfactorily without the addition of vitamins. A recent report by Gough and Lilly (87) supports this conclusion for T. viride.

The vitamin nutrition of fungi has been the subject of considerable study since Schopfer (179) demonstrated that thiamine was essential to the growth of Phycomyces. The literature on the subject has been adequately reviewed by Burkholder (34), Fries (68), Lilly and Barnett (124), Robbins and Kavanagh (171) and Schopfer (186).

Seven substances which have been shown to possess vitamin activity

for fungi were used in this study.

Thiamine.

Thiamine, or one of its components (pyrimidine and thiazole), has been shown to be essential for the growth of a large number of fungi, notably members of the Basidiomycetes (69, 148). White (232) reported that it was required by *O. graminis*, and its essential nature for the growth of *F. annosus* was demonstrated by Rennerfelt (162) and confirmed by Fries (148) and Jennison et al. (110).

Pyridoxine.

There have been comparatively few demonstrations of pyridoxine requirements among fungi.

It has been reported as essential for certain Ascomycetes, notably species of *Ceratostomella* (67, 172), for *Graphium ulmi* (35), and for *Trichophyton discoides* (174).

Pantothenic acid.

Requirements for pantothenic acid have been found chiefly among the yeasts (235), although a report by Wooster and Cheldelin (244) indicating a pantothenic acid deficiency in *Penicillium digitatum*, and one by Tatum and Beadle (197) of a mutant of *Neurospora* requiring this substance, suggest that pantothenic acid requirements may exist among the filamentous fungi.

Biotin.

Biotin has been shown to be essential for a number of fungi, mostly Ascomycetes, the yeasts in general being deficient (34). Kogl and Fries (113) were probably the first to demonstrate a biotin requirement among filamentous fungi when they showed that *Nematospora gossypii* did

not grow in its absence. Fries (67) and others have demonstrated biotin deficiencies in fungi. Padwick (154) observed the necessity of growth factors present in certain plant or animal extracts for the growth of O. graminis on synthetic media. White (232) demonstrated that O. graminis in addition to thiamine, mentioned above, also required biotin for growth, and this was confirmed by Gilpatrick (79).

Biotin is active in very much lower amounts than most of the other vitamins and is commonly incorporated into culture media at one tenth of the concentration of thiamine, for example.

Inositol.

It is questionable whether i-inositol may be regarded as a true vitamin (Cantarow and Schepartz (40)) as it is active in larger amounts and occurs in plant and animal tissues in greater quantities than the vitamins considered. According to the same authors no catalytic function has been demonstrated for inositol. Nevertheless, its importance has been demonstrated for several fungi, e.g. Kogl and Fries (113), Fries (66), Robbins et al. (174), Leonian and Lilly (119), Burkholder (34), and consequently it was considered reasonable to include it in the present study.

Niacinamide.

Deficiencies for niacin are very common among the yeasts (34), but appear to be rare among filamentous fungi.

p-Aminobenzoic acid.

Rhodotorula aurantica was shown to be deficient for p-aminobenzoic acid by Robbins and Ma (173); small concentrations were found to be active. Deficiencies among filamentous fungi have only been reported for artificially

produced mutants (68).

Both niacinamide and p-aminobenzoic acid were included chiefly for the sake of completeness. Certain other compounds of doubtful vitamin status have been shown to have growth stimulating effects (e.g. choline, adenine and several amino acids (148)) but are not considered here.

MATERIALS AND METHODS.

As stated earlier in considering the methods used in nutritional studies the use of agar may be expected to introduce certain unknown substances into the medium (170). Observations of O. graminis and F. annosus considered in the previous section, however, indicated that the grade of agar used in these experiments contained vitamins, if any, in amounts too small to interfere with the demonstration of vitamin deficiencies in these two fungi. Therefore, an agar solidified basic medium was used in the following experiment. Asparagine was chosen as nitrogen source as it was the only one which was observed to support satisfactory growth of both species.

The basic medium was, therefore, as follows:

Glucose	12.51 grams
L-Asparagine	2.36 grams
KH_2PO_4	1.00 grams
$MgSO_4.7H_2O$	0.50 grams
Fe ion, Zn ion, Mn ion.	
Agar	17.50 grams
Distilled water to make 1 litre	

To determine which of the seven vitamins were essential for growth they were added to the basic medium in 16 different combinations as indicated in Table 11. The concentrations used were the same as those described under the basic medium, namely:

Thiamine hydrochloride	100 micrograms per litre
Pyridoxine hydrochloride	100 micrograms per litre
Calcium pantothenate	100 micrograms per litre
Biotin	10 micrograms per litre
i-Inositol	5 milligrams per litre
Niacinamide	100 micrograms per litre
p-Aminobenzoic acid	100 micrograms per litre

The pH of the media was adjusted to 5.5 after addition of the vitamins.

Preparation of the Petri dishes and inoculation was carried out as in previous experiments. The inoculum was grown on the basic medium, supplemented with the complete vitamin mixture. Transfer of some vitamins with the inoculum was, therefore, unavoidable, and presumably accounted for the growth observed on the control medium. Four Petri dishes for each treatment were inoculated with each fungus.

Growth in diameter was measured after the incubation times given in Table 11.

RESULTS.

The results are given in Table 11 as the mean diameter in millimetres of the four colonies for each treatment.

For *O. graminis* it is apparent that omission of either thiamine or

TABLE 11. THE EFFECT OF VITAMINS ON THE GROWTH OF *OPHIOBOLUS GRAMINIS* AND *FOMES ANNOSUS*.

		GROWTH AS MEAN DIAMETER OF FUNGUS COLONY IN MILLIMETRES.	
		OPHIOBOLUS GRAMINIS [1]	*FOMES ANNOSUS* [1]
1.	All vitamins	74.25	72.50
2.	All vitamins except thiamine	17.25	36.66 [2] [3]
3.	All vitamins except pyridoxine	71.00	59.50
4.	All vitamins except pantothenic acid	75.50	66.75
5.	All vitamins except biotin	31.25	71.66 [2]
6.	All vitamins except inositol	73.50	70.50
7.	All vitamins except niacinamide	72.50	72.33 [2]
8.	All vitamins except *p*-Aminobenzoic acid	75.50	67.00
9.	Thiamine alone	30.00	63.75
10.	Pyridoxine alone	15.50	39.66 [2] [3]
11.	Pantothenic acid alone	17.33 [2]	35.75 [3]
12.	Biotin alone	16.00 [2]	36.50 [3]
13.	Inositol alone	15.00	39.50 [3]
14.	Niacinamide alone	14.50	35.75 [3]
15.	p-Aminobenzoic acid alone	14.50	37.00 [3]
16.	Control (no vitamins)	19.75	38.50 [3]

---

[1] 10 days growth.

[2] Mean of 3 plates; all other values mean of 4 plates.

[3] Very light starvation type growth.

biotin resulted in a marked reduction in growth. Omission of thiamine alone produced the greatest effect, growth being reduced to that of the control; when added alone, that is in spite of biotin deficiency, it did cause increase in growth over the control. Omission of biotin from the complete mixture reduced growth very close to that with thiamine alone, while biotin alone did not increase growth over the control. These results, therefore, indicate that of the seven vitamins used here, O. graminis is deficient for thiamine and biotin. None of the others, either by omission from the complete mixture or addition separately, produced any distinct effect. The results of other workers using different isolates are, therefore, confirmed here (79, 232).

F. annosus demonstrated a clear-cut deficiency for thiamine, and possibly a partial deficiency for pyridoxine. This was indicated by the fact that omission of thiamine reduced growth to that of the control but addition of thiamine alone to the basic medium did not increase growth to that on the complete mixture. Omission of pyridoxine reduced growth to a level similar to that given by thiamine alone.

Thus in its thiamine deficiency this isolate of F. annosus is similar to those studied by other investigators (69, 110, 162).

Figure 7 illustrates the comparative growth of these two fungi with and without added vitamins.

Thus the two parasites studied in this investigation both required thiamine for growth. O. graminis also required biotin, and growth of F. annosus was possibly further stimulated by pyridoxine. Neither saprophyte required any vitamin supplement for full growth.

The possibility that different growth-factor requirements might have

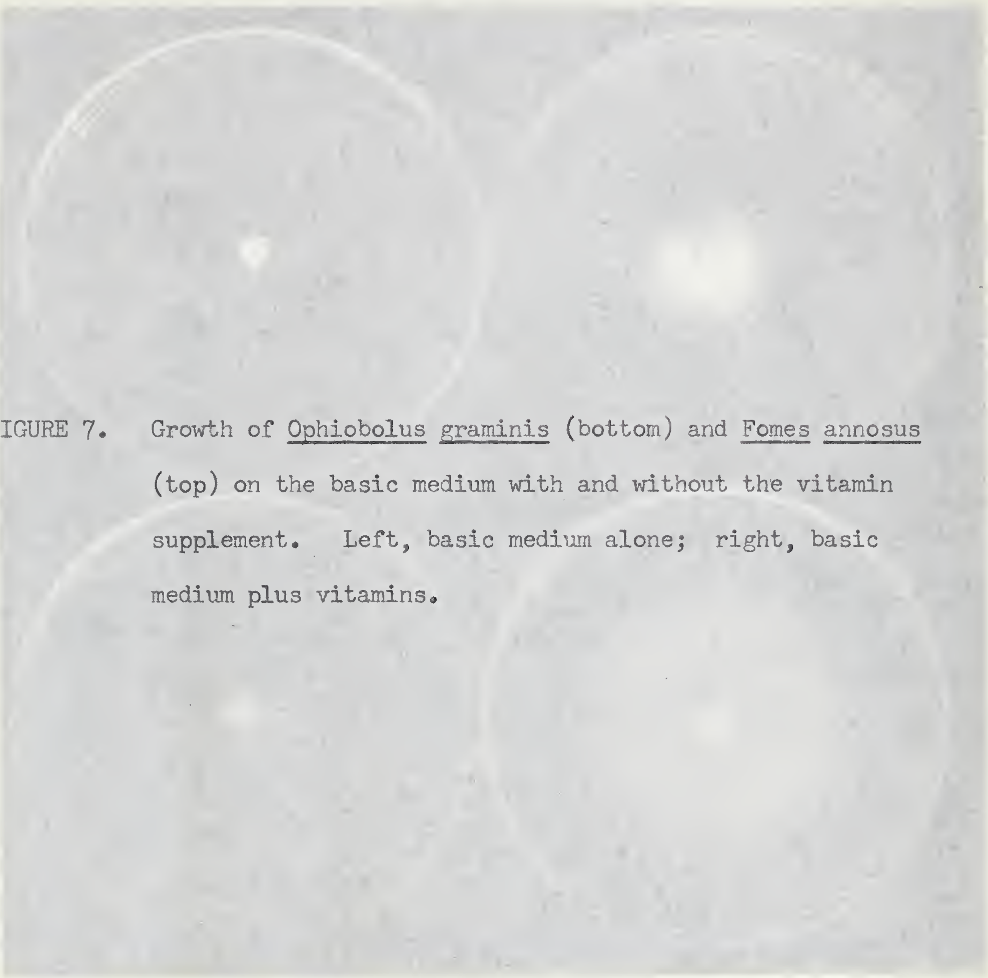
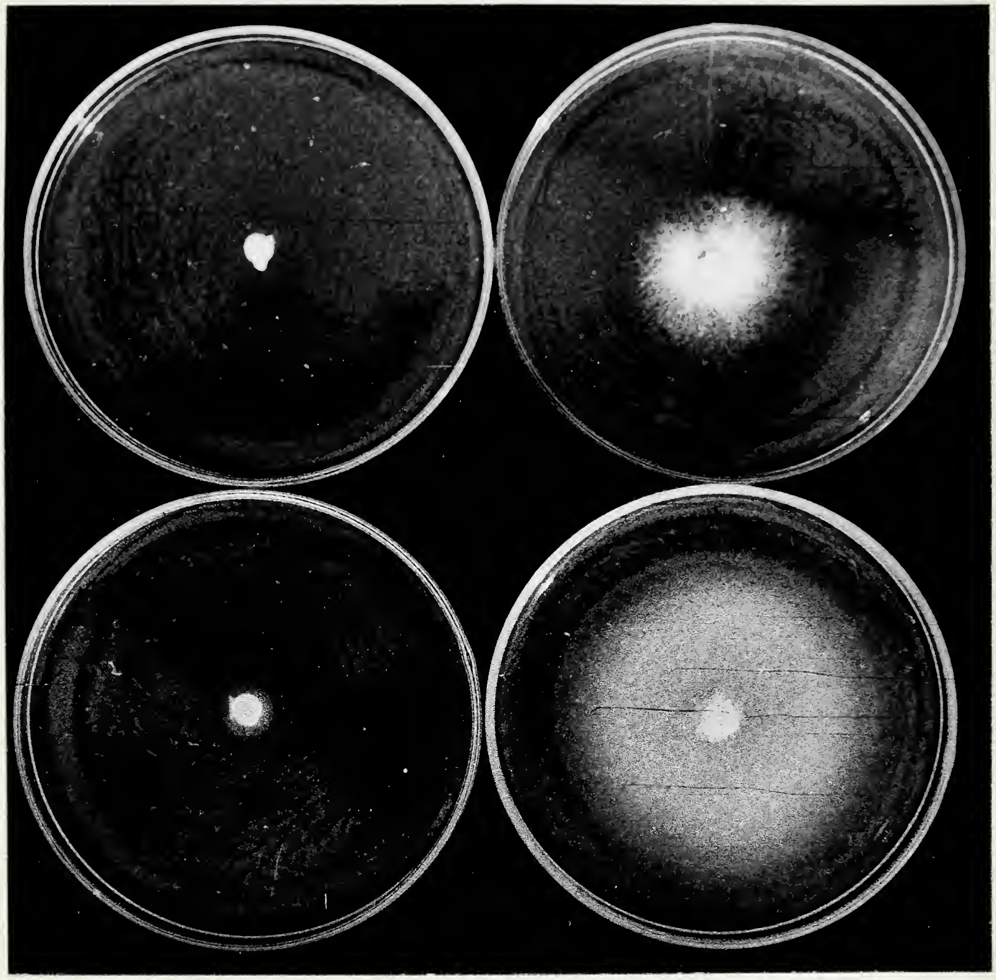


FIGURE 7. Growth of Ophiobolus graminis (bottom) and Fomes annosus (top) on the basic medium with and without the vitamin supplement. Left, basic medium alone; right, basic medium plus vitamins.



significance from an ecological point of view has been discussed by Fries (68). Attractive as this possibility might seem, Fries points out that thiamine deficiencies have been found among saprophytes, parasites and mycorrhiza formers. The clear-cut distinction between saprophytes and parasites with regard to vitamins reported here would appear to be mere coincidence. This will, however, be discussed further subsequently in this report.

UTILIZATION OF VARIOUS CARBOHYDRATES BY _TRICHODERMA VIRIDE_, _TRICHOCLADIUM ASPERUM_, _OPHIOBOLUS GRAMINIS_ AND _FOMES ANNOSUS_.

A consideration of all the carbon compounds which may act as carbon sources for fungi would be impracticable. Here an attempt is made to compare growth on a few representatives of one of the more important groups, the carbohydrates, which are probably the major source of carbon for fungi.

MATERIALS AND METHODS.

The basic medium considered earlier was used throughout the following experiments, substitutions of the various carbohydrates being made for glucose, which then served as a standard of comparison. The medium had the composition:

Carbon source	equivalent to 5.0 grams of carbon
Asparagine	2.36 grams
KH_2PO_4	1.00 grams
$MgSO_4.7H_2O$	0.50 grams

Fe ion, Zn ion, Mn ion

Vitamin mixture

Distilled water to make 1 litre

Media solidified with agar proved unsatisfactory in preliminary studies, for differences in density of growth were frequently much greater than differences in diameter. Therefore the experiments were carried out in culture flasks using 20 ml. of medium per flask. Usually 6 flasks for each carbon source were inoculated with each fungus and where possible data were collected from five, and a statistical analysis was carried out.

The inoculum was grown on the basic medium with glucose as carbon source and consisted of 5 m.m. plugs from the edge of the cultures. The plugs were supported on the bent tips of glass rods to prevent them being submerged.

The mycelium was harvested at different times in the various experiments depending on the carbohydrates used. An attempt was made to harvest a particular experiment when growth on any one sugar reached the edge of the flask. In this way, and by comparing all data to growth on the glucose control, it was hoped to obtain as relative results as possible.

The carbohydrates were considered in four groups.

HEXOSE MONOSACCHARIDES.

Hexoses have probably been studied to a greater degree than any other group with regard to utilization by fungi. The following hexoses are stated to occur naturally (124): D-glucose, D-fructose, D-mannose, D-galactose, L-galactose and L-sorbose. Steinberg (189) found D-glucose,

D-fructose, D-mannose and L-sorbose to be of equal value for the nutrition of *Aspergillus niger* and D-galactose to be poor. Mosher et al. (139) found D-glucose, D-mannose and D-galactose to give similar growth of *Trichophyton interdigitale* and D-fructose somewhat less. Tamiya (196), according to Lilly and Barnett (124), reported mannose to be the most favourable hexose for growth of *Aspergillus oryzae*. Growth of *Melanospora destruens* was similar on D-glucose, D-fructose and D-mannose, (Hawker (96)), but D-galactose did not support growth. Although D-galactose is a poor carbon source for many fungi it has been reported as satisfactory for *Rhizopus suinus* (44), *Rhizoctonia* (135), as well as for *T. interdigitale* mentioned above. Lilly and Barnett (125) found D-galactose to be used well by many fungi after an initial lag. Norkrans (148) found D-galactose to be a poor carbon source for various species of *Tricholoma*; considerable variation was noticed in the utilization of the other hexoses, particularly of D-fructose. White (233) found glucose to provide optimum conditions for growth of *O. graminis*, while Gilpatrick (79) found growth on D-glucose, D-fructose and D-mannose to be similar and better than that on D-galactose.

In the present investigation the growth of *T. viride*, *T. asperum*, *O. graminis* and *F. annosus* was observed on D-glucose, D-fructose, D-mannose and D-galactose.

RESULTS.

The results are presented in Table 12.

For *T. viride* fructose is apparently the best carbon source, growth being significantly better than on the other three hexoses. D-galactose

TABLE 12. COMPARATIVE GROWTH OF *TRICHODERMA VIRIDE*, *TRICHOCLADIUM ASPERUM*, *OPHIOBOLUS GRAMINIS* AND *FOMES ANNOSUS* ON GLUCOSE, FRUCTOSE, MANNOSE AND GALACTOSE.

a) *TRICHODERMA VIRIDE*.

		MILLIGRAMS DRY MYCELIUM PER FLASK					TOTAL	MEAN
8 days growth	Glucose	100.9	103.8	97.9	95.5	91.4	489.5	97.9
	Fructose	110.9	102.1	110.9	103.4	108.1	535.4	107.1
	Mannose	97.7	94.7	91.9	93.5	95.5	473.3	94.7
	Galactose	88.5	84.2	85.6	88.2	92.3	438.8	87.8

ANALYSIS OF VARIANCE.

SOURCE OF VARIATION.	D.F.	M.S.	F.	F. at 1%	L.S.D. at 1%
Treatments	3	321.96	23.63	5.29	6.82
Error	16	13.63			
Total	19				

TABLE 12 (CONTINUED).

b) *TRICHOCLADIUM ASPERUM.*

		MILLIGRAMS DRY MYCELIUM PER FLASK					TOTAL	MEAN
12 days growth	Glucose	137.6	120.6	123.4	120.1	126.5	628.2	125.6
	Fructose	133.6	143.0	132.1	138.7	130.9	678.3	135.7
	Mannose	116.5	122.0	126.2	129.7	123.5	617.9	123.6
	Galactose	74.9	107.2	86.5	101.0	120.9	490.5	98.1

ANALYSIS OF VARIANCE.

SOURCE OF VARIATION	D.F.	M.S.	F.	F. at 1%	L.S.D. at 1%
Treatments	3	1278.73	12.12	5.29	18.98
Error	16	105.53			
Total	19				

TABLE 12 (CONTINUED).

c) *OPHIOBOLUS GRAMINIS.*

		MILLIGRAMS DRY MYCELIUM PER FLASK					TOTAL	MEAN
11 days growth	Glucose	145.4	142.5	138.9	136.7	145.1	708.6	141.7
	Fructose	152.2	141.1	143.3	146.2	147.0	729.8	145.9
	Mannose	147.7	137.0	145.8	135.8	139.4	705.7	141.1
	Galactose	109.6	101.7	104.6	93.6	114.0	523.5	104.7

ANALYSIS OF VARIANCE.

SOURCE OF VARIATION	D.F.	M.S.	F.	F. at 1%	L.S.D. at 1%
Treatments	3	1850.94	60.97	5.29	10.18
Error	16	30.36			
Total	19				

TABLE 12 (CONTINUED).

d) FOMES ANNOSUS.

		MILLIGRAMS DRY MYCELIUM PER FLASK					TOTAL	MEAN
12 days growth	Glucose	64.1	72.2	56.3	70.3	60.7	333.6	64.7
	Fructose	85.4	73.9	67.2	73.9	69.4	369.8	73.9
	Mannose	79.1	70.2	76.7	71.3	75.9	373.2	74.6
	Galactose	51.5	48.2	63.6	42.2	52.4	257.9	51.6

ANALYSIS OF VARIANCE.

SOURCE OF VARIATION.	D.F.	M.S.	F.	F. at 1%	L.S.D. at 1%
Treatments	3	578.97	13.76	5.29	11.98
Error	16	42.07			
Total	19				

supported the least growth. In preliminary experiments on solid media it had been observed that L-sorbose did not support growth of this fungus.

The results with T. asperum appeared similar to those with T. viride, D-galactose again being the poorest carbon source. This fungus was able to grow well on L-sorbose on solid media.

Both O. graminis and F. annosus grew poorly on D-galactose and growth on D-glucose, D-fructose and D-mannose was similar. Growth of F. annosus on D-fructose and D-mannose appeared better than on D-glucose but the differences were not quite significant. Neither of these fungi made appreciable growth on L-sorbose. The observations made here for O. graminis support those of previous investigators.

Thus all four fungi appear similar in their ability to use hexose sugars with the exception of T. asperum which made good growth on L-sorbose.

PENTOSE MONOSACCHARIDES.

There have been comparatively few investigations of the utilization of pentoses by fungi.

Steinberg (190) compared the growth of Aspergillus niger on a number of pentoses, namely: D-lyxose, D-xylose, L-xylose, D-arabinose, L-arabinose, D-ribose and L-ribose. Satisfactory growth occurred only on D-xylose and L-arabinose. Wolf (240) found that D-arabinose did not support growth of Ustilago zeae but L-arabinose and D-xylose did. Lilly and Barnett (125) investigated the growth of a variety of fungi on D-xylose and L-arabinose; D-xylose was usually the better carbon source. Phytophthora infestans did not grow on either D-xylose or L-arabinose, Schizothecium longicolle grew only slightly on L-arabinose and Thielaviopsis

basicola did not utilize D-xylose. Lilly and Barnett (126) compared the growth of a number of fungi on D- and L-arabinose. Of the two, L-arabinose supported better growth in all but one case, being occasionally as good or better than glucose. Only three of seven species of *Tricholoma* tested by Norkrans (148) grew satisfactorily on D-xylose.

Gilpatrick (79) found D-xylose slightly inferior to D-glucose as a carbon source for *O. graminis*. No other references to the utilization of pentoses by the fungi studied here have been found by the author.

Bonner (12) states that L-arabinose, D-xylose and D-ribose are the most widely distributed pentoses in nature. In addition to these three, D-arabinose has also been included in the present investigation.

RESULTS.

The results are presented in Table 13. Figure 8 illustrates the growth of *T. asperum* and *F. annosus* on the various pentoses.

D-Arabinose was poorly utilized by all four fungi, and for *T. viride* and *F. annosus* growth was not significantly better on L-arabinose. For *O. graminis* and *T. asperum* growth on L-arabinose was quite good although poorer than on glucose.

Utilization of D-ribose was variable. *T. asperum* made very poor growth with this sugar. *F. annosus* and *O. graminis* made significantly less growth than with glucose and *T. viride* also made less but it was not significantly so.

Growth of all the fungi on D-xylose appeared reduced compared with that on D-glucose, but apparently only for *O. graminis* was the difference significant.

TABLE 13. COMPARATIVE GROWTH OF *TRICHODERMA VIRIDE*, *TRICHOCLADIUM ASPERUM*, *OPHIOBOLUS GRAMINIS* AND *FOMES ANNOSUS* ON D-GLUCOSE, L-ARABINOSE, D-ARABINOSE, D-RIBOSE AND D-XYLOSE.

a) *TRICHODERMA VIRIDE*.

		MILLIGRAMS DRY MYCELIUM PER FLASK					TOTAL	MEAN
10 days growth	D-glucose	85.5	89.0	85.0	79.6	88.3	427.4	85.5
	L-arabinose	35.9	28.1	34.7	31.7	33.8	164.2	32.8
	D-arabinose	25.0	27.5	28.9	34.3	28.7	144.4	28.9
	D-ribose	76.4	77.9	78.6	77.0	73.1	383.0	76.6
	D-xylose	72.2	74.3	82.8	78.3	72.7	380.3	76.1

ANALYSIS OF VARIANCE.

SOURCE OF VARIATION.	D.F.	M.S.	F.	F. at 1%	L.S.D. at 1%
Treatments	4	3611.04	304.86	4.43	27.69
Error	20	11.85			
Total	24				

TABLE 13 (CONTINUED)

b) *TRICHOCLADIUM ASPERUM.*		MILLIGRAMS DRY MYCELIUM PER FLASK					TOTAL	MEAN
10 days growth	D-glucose	142.4	136.7	134.3	134.3	126.9	674.6	134.9
	L-arabinose	80.9	93.1	92.0	101.7	84.3	452.0	90.4
	D-arabinose	41.8	36.9	31.2	28.1	16.2	154.2	30.8
	D-ribose	27.2	29.5	41.1	34.0	43.2	175.0	35.0
	D-xylose	121.0	127.1	131.6	117.5	111.6	608.8	121.8

ANALYSIS OF VARIANCE.

SOURCE OF VARIATION.	D.F.	M.S.	F.	F. at 1%	L.S.D. at 1%
Treatments	4	11595.72	191.36	4.43	14.01
Error	20	60.60			
Total	24				

TABLE 13 (CONTINUED).

c) OPHIOBOLUS GRAMINIS.		MILLIGRAMS DRY MYCELIUM PER FLASK					TOTAL	MEAN
14 days growth	D-glucose	117.5	132.2	138.1	135.0	141.5	655.3	131.1
	L-arabinose	120.8	117.0	99.4	103.5	112.4	553.1	110.6
	D-arabinose	68.0	61.5	68.3	57.2	53.6	308.6	61.7
	D-ribose	95.2	108.1	118.2	95.8	93.3	510.6	102.1
	D-xylose	99.0	98.5	120.5	124.9	116.6	559.5	111.9

ANALYSIS OF VARIANCE.

SOURCE OF VARIATION.	D.F.	M.S.	F.	F. at 1%	L.S.D. at 1%
Treatments	4	3285.34	33.12	4.43	17.92
Error	20	99.20			
Total	24				

TABLE 13 (CONTINUED).

d) *FOMES ANNOSUS*.

		MILLIGRAMS DRY MYCELIUM PER FLASK					TOTAL	MEAN
14 days growth	D-glucose	75.8	66.0	61.9	65.3	82.4	351.4	70.3
	L-arabinose	55.4	43.6	53.7	39.8	32.1	224.6	44.9
	D-arabinose	32.8	33.6	40.0	41.3	37.8	185.5	37.1
	D-ribose	43.6	53.1	52.5	39.2	28.1	216.5	43.3
	D-xylose	56.0	46.6	68.0	71.2	60.6	302.4	60.5

ANALYSIS OF VARIANCE.

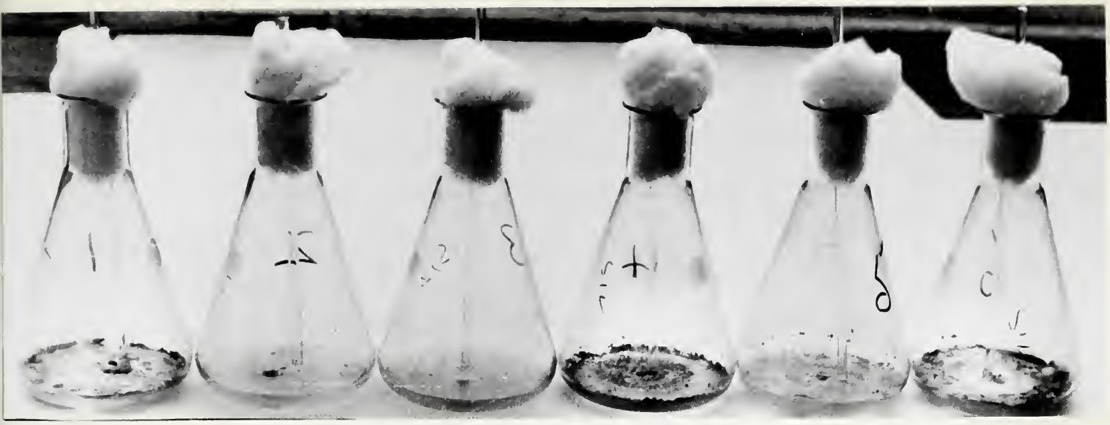
SOURCE OF VARIATION.	D.F.	M.S.	F.	F. at 1%	L.S.D. at 1%
Treatments	4	938.53	12.19	4.43	17.92
Error	20	77.00			
Total	24				

FIGURE 8. Growth of *Trichoderma viride* (top), *Trichocladium asperum*, (centre) and *Fomes annosus* (bottom) on various carbohydrates.

Top. *Trichoderma viride* on (left to right) D-glucose, maltose, sucrose, lactose, melibiose, cellobiose.

Centre. *Trichocladium asperum* on (left to right) D-glucose, L-arabinose, D-arabinose, D-ribose, D-xylose.

Bottom. *Fomes annosus* on (left to right) D-glucose, L-arabinose, D-arabinose, D-ribose, D-xylose.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Reference to the summary table (Table 17) or the chart (Figure 9) indicates that utilization of D- and L-arabinose by the parasites was somewhat better than by the saprophytes. Utilization of D-xylose was much the same, while that of D-ribose varied.

DISACCHARIDES.

Various disaccharides (notably sucrose) are widely distributed and of considerable metabolic importance in higher plants. They must, therefore, form potential substrate material for saprophytic and parasitic fungi. A study of the relative ability of *T. viride*, *T. asperum*, *O. graminis* and *F. annosus* to utilize these compounds for growth is, therefore, warranted.

References to systematic observations on the utilization of disaccharides by fungi are few. Norkrans (148) found growth of all but one of seven species of *Tricholoma* to be poor on lactose. Two grew satisfactorily on maltose, and three similarly on sucrose. Beckman et al. (5) found *Chalara quercina* made good growth on cellobiose, maltose and sucrose, but poor growth on lactose and melibiose. Wolf (240) found sucrose, maltose and lactose to be adequately utilized by *Ustilago zeae* but cellobiose and particularly melibiose supported somewhat less growth. Lilly and Barnett (125) found considerable differences to exist between the fungi they studied with regard to utilization of disaccharides. Hofsten (103) found maltose and sucrose to be rapidly utilized by *Ophiostoma multiannulatum*. Craseman (45) found two species of *Blastocladia* to differ in their ability to utilize disaccharides. *B. ramosa* grew on maltose but not on sucrose, but the reverse was true of *B. pringsheimii*.

Golueke (83) observed Sapromyces elongatus and Apodachyla sp. to make as much growth on sucrose as on glucose. Vining and Taber (209) found Claviceps purpurea to utilize cellobiose and sucrose readily but not maltose.

Of the fungi used here Gilpatrick (79) obtained best growth of O. graminis on maltose. That on lactose and sucrose was somewhat poorer.

Five disaccharides were used in the present investigation: maltose, sucrose, lactose, melibiose and cellobiose.

RESULTS.

The results are given in Table 14. Figure 8 illustrates the growth of T. viride on the disaccharides.

Again there appear to be no consistent differences distinguishing the two saprophytes from the parasites. For T. viride only growth on sucrose was significantly different from that on D-glucose. Growth of T. asperum was significantly poorer on lactose and melibiose than on glucose or the other sugars. This might have been expected from the relatively poor growth of this fungus on galactose.

Growth of O. graminis was poor on melibiose and on maltose and just significantly less than that on glucose. The latter finding is contrary to that of Gilpatrick (79) who observed maltose to be a better carbon source than glucose.

With F. annosus only cellobiose supported significantly less growth than D-glucose.

The significance of these observations with respect to the enzymes involved in utilization of these sugars will be discussed shortly.

TABLE 14. COMPARATIVE GROWTH OF *TRICHODERMA VIRIDE*, *TRICHOCLADIUM ASPERUM*, *OPHIOBOLUS GRAMINIS* AND *FOMES ANNOSUS* ON D-GLUCOSE, MALTOSE, SUCROSE, LACTOSE, MELIBIOSE AND CELLOBIOSE.

a) *TRICHODERMA VIRIDE.*

		MILLIGRAMS DRY MYCELIUM PER FLASK				TOTAL	MEAN
10 days growth	D-Glucose	84.7	73.7	108.6	89.2	356.2	89.1
	Maltose	64.3	86.9	59.6	67.8	278.6	69.7
	Sucrose	42.4	51.9	40.2	68.6	203.1	50.8
	Lactose	114.8	104.0	93.9	72.3	385.0	96.3
	Melibiose	115.3	82.4	123.7	72.5	393.9	98.5
	Cellobiose	113.5	94.7	111.3	123.1	442.6	110.7

ANALYSIS OF VARIANCE.

SOURCE OF VARIATION.	D.F.	M.S.	F.	F. at 1%	L.S.D. at 1%
Treatments	5	1909.41	7.12	4.25	33.31
Error	18	267.89			
Total	23				

TABLE 14 (CONTINUED).

b) TRICHOCLADIUM ASPERUM.

		MILLIGRAMS DRY MYCELIUM PER FLASK				TOTAL	MEAN
10 days growth	D-Glucose	131.3	129.6	121.6	133.8	516.3	129.1
	Maltose	126.5	130.0	133.0	136.0	525.5	131.4
	Sucrose	131.9	135.7	129.3	133.0	529.9	132.5
	Lactose	71.1	79.3	53.8	57.7	252.9	63.2
	Melibiose	44.8	39.6	43.6	57.9	185.9	46.5
	Cellobiose	130.0	128.1	143.9	141.2	543.2	135.8

ANALYSIS OF VARIANCE.

SOURCE OF VARIATION.	D.F.	M.S.	F.	F. at 1%	L.S.D. at 1%
Treatments	5	6509.80	153.72	4.25	13.24
Error	18	42.35			
Total	23				

TABLE 14 (CONTINUED).

c) OPHIOBOLUS GRAMINIS.

		MILLIGRAMS DRY MYCELIUM PER FLASK				TOTAL	MEAN
10 days growth	D-Glucose	127.7	107.6	126.6	115.3	477.2	119.3
	Maltose	83.2	77.4	105.9	110.2	376.7	94.2
	Sucrose	114.5	111.2	116.6	115.9	458.2	114.6
	Lactose	128.0	120.0	142.7	145.3	545.9	136.5
	Melibiose	70.3	51.3	38.6	39.0	199.2	49.8
	Cellobiose	132.9	137.4	124.8	144.5	539.6	134.9

ANALYSIS OF VARIANCE.

SOURCE OF VARIATION.	D.F.	M.S.	F.	F. at 1%	L.S.D. at 1%
Treatments	5	4226.53	34.72	4.25	22.45
Error	18	121.71			
Total	23				

TABLE 14 (CONTINUED).

d) *FOMES ANNOSUS.*

		MILLIGRAMS DRY MYCELIUM PER FLASK				TOTAL	MEAN
15 days growth	D-Glucose	92.6	94.1	94.0	87.6	368.3	92.1
	Maltose	88.4	92.5	95.3	107.7	383.0	96.0
	Sucrose	87.8	80.4	72.2	80.4	320.8	80.2
	Lactose	90.1	88.7	86.7	85.7	351.2	87.8
	Melibiose	67.0	80.8	73.3	75.9	297.0	73.3
	Cellobiose	56.4	63.2	62.4	63.1	245.1	61.3

ANALYSIS OF VARIANCE.

SOURCE OF VARIATION.	D.F.	M.S.	F.	F. at 1%	L.S.D. at 1%
Treatments	5	658.59	23.70	4.25	10.73
Error	18	27.79			
Total	23				

POLYSACCHARIDES.

Polysaccharides form the major structural and storage constituents of higher plants. Invasion of higher plant cells by fungi involves the penetration of cell walls, the major components of which are pectin and cellulose. Subsequent decomposition of dead plant material in the soil must also involve the breakdown of these substances and of lignin.

It is generally conceded that comparatively few fungi are able to attack the more complex molecules of the polysaccharides and, in consequence of their complexity, make somewhat slower growth than on their component molecules.

Because of their association with trees much attention has been given to the ability of basidiomycetes to decompose cellulose. Nobles (146) lists many fungi which are able to decompose cellulose, and Nord and Vitucci (147) have reviewed the literature on the microbial breakdown of cellulose.

Norkrans (148) found three species of *Tricholoma* out of seven to grow on cellulose, three also used starch and two used inulin. Wolf (240) did not obtain any growth of *Ustilago zeae* on starch. Beckman et al. (5) found cellulose, inulin, pectin and starch to be very poor sources of carbon for *Chalara quercina*. Vining and Taber (209) reported no appreciable growth of *Claviceps purpurea* on cellulose or starch.

Thaysen and Bunker (198) report that van Iterson (107) observed cellulose destruction by *T. asperum*. *T. viride* has also been frequently reported as attacking cellulose (e.g. Mandels (132)).

Four polysaccharides were considered in the following experiment: inulin, starch, pectin and cellulose.

Because of the insoluble nature of cellulose and the fact that the experiments were carried out in still cultures a different method of inoculation of the flasks was used than hitherto. Instead of plugs from agar plates a homogenate of the mycelium in 0.25 M sodium chloride was used, 2 ml. of homogenate being added per flask. The method is described in more detail in Part III of this report.

The insoluble nature of the cellulose powder also necessitated weighing the required amount separately into each culture flask rather than preparing it in solution with the rest of the medium.

RESULTS.

The data for cellulose are recorded in Table 15; for the other polysaccharides in Table 16.

TABLE 15. COMPARATIVE GROWTH OF *TRICHODERMA VIRIDE*, *TRICHOCLADIUM ASPERUM*, *OPHIOBOLUS GRAMINIS* AND *FOMES ANNOSUS* ON D-GLUCOSE AND CELLULOSE.

	TRICHODERMA VIRIDE	*TRICHOCLADIUM ASPERUM*	*OPHIOBOLUS GRAMINIS*	*FOMES ANNOSUS*
D-Glucose	M	M	M	M
Cellulose	-	-	-M	M

M Indicates formation of typical surface mat.

TABLE 16. COMPARATIVE GROWTH OF TRICHODERMA VIRIDE, TRICHOCLADIUM ASPERUM, OPHIOBOLUS GRAMINIS AND FOMES ANNOSUS ON D-GLUCOSE, INULIN, STARCH AND PECTIN.

a) TRICHODERMA VIRIDE.

		MILLIGRAMS DRY MYCELIUM PER FLASK				TOTAL	MEAN
10 days growth	D-Glucose	85.9	92.0	96.3	88.8	364.0	91.0
	Inulin	44.9	43.1	44.2	51.8	184.0	46.0
	Starch	119.2	116.6	113.5	82.6	431.9	108.0
	Pectin	68.5	70.4	72.6	65.6	277.1	69.3

ANALYSIS OF VARIANCE.

SOURCE OF VARIATION.	D.F.	M.S.	F.	F. at 1%.	L.S.D. at 1%
Treatments	3	2888.48	32.28	5.95	20.43
Error	12	89.47			
Total	15				

TABLE 16 (CONTINUED).

b) TRICHOCLADIUM ASPERUM.

		MILLIGRAMS DRY MYCELIUM PER FLASK				TOTAL	MEAN
10 days growth	D-Glucose	129.7	132.3	127.5	116.7	506.2	126.6
	Inulin	126.6	128.4	129.7	122.5	507.2	126.8
	Starch	130.5	120.7	133.5	115.3	500.0	125.0
	Pectin	99.7	100.7	98.6	94.5	393.5	98.4

ANALYSIS OF VARIANCE.

SOURCE OF VARIATION.	D.F.	M.S.	F.	F. at 1%	L.S.D. at 1%
Treatments	3	772.14	22.73	5.95	12.59
Error	12	33.98			
Total	15				

TABLE 16 (CONTINUED).

c) OPHIOBOLUS GRAMINIS.

		MILLIGRAMS DRY MYCELIUM PER FLASK				TOTAL	MEAN
14 days growth	D-Glucose	148.4	148.2	134.0	144.2	574.8	143.7
	Inulin	77.3	77.5	74.8	78.0	307.6	76.9
	Starch	134.2	126.8	122.7	121.6	505.3	126.3
	Pectin	114.3	101.4	97.7	88.6	402.0	100.5

ANALYSIS OF VARIANCE.

SOURCE OF VARIATION.	D.F.	M.S.	F.	F. at 1%	L.S.D. at 1%
Treatments	3	3432.36	70.86	5.95	15.03
Error	12	48.44			
Total	15				

TABLE 16 (CONTINUED).

d) *FOMES ANNOSUS.*

		MILLIGRAMS DRY MYCELIUM PER FLASK				TOTAL	MEAN
16 days growth	D-Glucose	105.8	96.9	97.6	100.9	401.2	100.3
	Inulin	65.8	70.1	49.3	49.2	234.4	58.6
	Starch	109.8	106.8	112.0	118.7	447.3	111.8
	Pectin	110.6	114.2	101.8	85.8	412.4	103.1

ANALYSIS OF VARIANCE.

SOURCE OF VARIATION.	D.F.	M.S.	F.	F. at 1%	L.S.D. at 1%
Treatments	3	2256.28	28.04	5.95	19.38
Error	12	80.46			
Total	15				

It proved impossible to separate the mycelium of the fungi from the cellulose and so dry weights of mycelium could not be determined. The differences between the fungi were, however, so marked that the method of recording the observations used in Table 15 is satisfactory.

Both O. graminis and F. annosus produced typical mats on the surface of the medium. No such growth occurred with T. viride and T. asperum.

With regard to the other polysaccharides, inulin gave poor growth except for T. asperum, for which it was quite satisfactory. T. viride and T. asperum produced significantly less mycelium on pectin than on glucose, as also did O. graminis. F. annosus grew as well on pectin as on glucose. Starch was utilized well by all fungi, although compared with the other species growth of O. graminis was not quite as good.

DISCUSSION AND CONCLUSIONS.

The results of the studies on carbohydrates are summarized in Table 17 and Figure 9, where the values are converted to percentages of the growth on the glucose control.

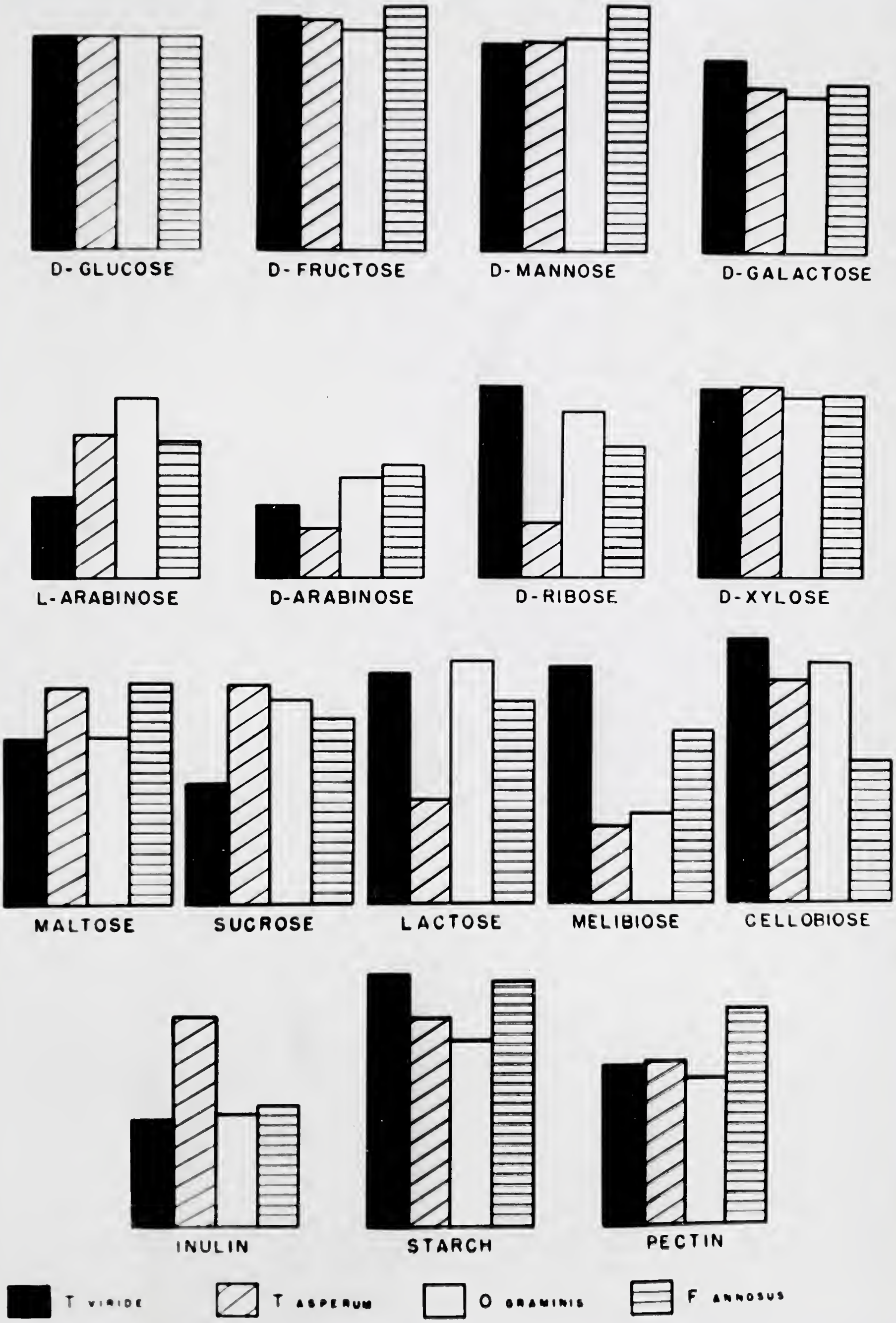
Ability of a fungus to utilize a monosaccharide as the main source of carbon, for growth, indicates that this substance can enter one of the main pathways of metabolism, either directly or after some modification. It must be able to serve as a source of energy in energy-yielding reactions and in addition must provide basic building material for synthesis of the structural elements and the protoplasmic components. Inability of a compound to serve in either one of these ways would result in lack of growth. Thus growth in itself can only give a positive indication of availability of a compound. It is quite possible that a sugar may be used as a

TABLE 17. GROWTH OF TRICHODERMA VIRIDE, TRICHOCLADIUM ASPERUM, OPHIOBOLUS GRAMINIS AND FOMES ANNOSUS ON ALL CARBOHYDRATES, AS PER CENT ON GLUCOSE.

	TRICHODERMA VIRIDE	TRICHOCLADIUM ASPERUM	OPHIOBOLUS GRAMINIS	FOMES ANNOSUS
D-Glucose	100.0	100.0	100.0	100.0
D-Fructose	109.4	108.0	103.0	114.3
D-Mannose	96.7	98.4	99.6	115.3
D-Galactose	89.6	78.1	73.9	79.7
L-arabinose	38.4	67.0	84.4	63.9
D-arabinose	33.8	22.9	47.1	52.8
D-ribose	89.6	25.9	77.9	61.6
D-xylose	89.0	90.2	85.4	86.1
Maltose	78.2	101.8	78.9	104.2
Sucrose	57.0	102.6	96.0	87.1
Lactose	108.1	48.0	114.4	95.4
Melibiose	110.6	26.0	41.7	80.6
Cellobiose	124.2	105.2	113.1	66.5
Inulin	50.5	100.2	53.5	58.4
Starch	118.6	98.8	87.9	111.5
Pectin	76.1	77.8	69.9	102.8
Cellulose	-	-	M	M

M Indicates formation of typical surface mat.

FIGURE 9. Diagramatic representation of the growth of *Trichoderma viride*, *Trichocladium asperum*, *Ophiobolus graminis* and *Fomes annosus* on various carbohydrates. All values are calculated as percentages of the growth on the glucose controls.


D-GLUCOSE
D-FRUCTOSE
D-MANNOSE
D-GALACTOSE
L-ARABINOSE
D-ARABINOSE
D-RIBOSE
D-XYLOSE
MALTOSE
SUCROSE
LACTOSE
MELIBIOSE
CELLOBIOSE
INULIN
STARCH
PECTIN
T VIRIDE
T ASPERUM
O GRAMINIS
F ANNOSUS

respiratory source and not for synthesis, and vice versa; either alone would be insufficient for growth but utilization might occur if the other requirement could be satisfied by a second substance.

It would most certainly be of interest, therefore, to carry out respiratory studies with some of the monosaccharides that have been found to support poor growth here. Hofsten (103) found that neither L-sorbose nor D-galactose supported growth of _Ophiostoma multiannulatum_ but both were quite rapidly respired. All the fungi studied grew considerably less well on galactose than on glucose. This could have been due to a lag in the synthesis of an adaptive enzyme as has been suggested by Lilly and Barnett (125), or due to the mechanism suggested above. Evidence to support the second possibility is to be found in the fact that except for _T. asperum_ growth on lactose (4-D-glucose beta-D-galactopyranoside) was as good as on D-glucose; growth of _F. annosus_ and _T. viride_ was also superior on melibiose (6-D-glucose alpha-D-galactopyranoside) to that on D-galactose. Both these disaccharides contain 1 molecule of D-galactose. Whether any adaptation to the utilization of D-galactose occurred could not be determined with the methods used in these experiments, but there is no obvious reason why adaptation should be any more rapid in the presence of a glucose molecule than in its absence. In fact, the literature on enzymic adaptation to D-galatose suggests the opposite to be true (186).

In the case of _T. asperum_ where negative results were obtained on all three substrates containing D-galactose it is impossible to determine if D-galactose utilization could occur. Lack of use could have been due to inability to produce the necessary hydrolytic enzymes to split the

disaccharides.

The same discussion may apply to the utilization of L-arabinose in which the configuration of the pyranose ring is identical to that of D-galactose. Lilly and Barnett (125) suggest that if configuration has anything to do with the rate of utilization of these two sugars then fungi able to use one should be able to use the other. It was not possible to confirm or refute this hypothesis here as all fungi grew comparatively poorly on both sugars.

The utilization of di- and poly-saccharides is rather more complicated. It is, of course, possible that the disaccharides at least may be utilized directly in the synthesis of more complex compounds, thus being utilized more economically than the corresponding monosaccharides. This might be suspected if growth on, for example, maltose or cellobiose was better than on glucose, but no significant increases in growth on these sugars over that on glucose were observed in these experiments. However, if utilization did occur in this way, breakdown of the molecule would also have to take place to provide substrates for respiration. Hence it is generally believed that utilization of di- and poly-saccharides involves first the hydrolysis of the larger molecules to monosaccharides before entering any of the main pathways of metabolism. Thus utilization of a di- or poly-saccharide will depend upon the ability of the fungus to secrete the necessary hydrolytic enzymes in addition to being able to use the constituent monosaccharides.

In the present study it is possible to infer that a fungus is able to hydrolyse a particular linkage when growth occurs on a di- or polysaccharide, but when growth is poor it is possible to conclude that the

required enzyme was lacking only if growth was good on the component monosaccharides.

The conclusions drawn are summarized in Table 18.

It is possible that all the enzymes required for the breakdown of starch are not produced by these fungi, hydrolysis of the 1-4 linkages would yield sufficient glucose for adequate growth. It is not obvious from the literature whether maltase is identical with one of the amylases, but it would seem reasonable to assume that it is. Ability to hydrolyse maltose would, therefore, permit growth on starch.

A further point of interest is that although cellobiase is produced by all four fungi, cellulase which hydrolyses the same linkage was produced here only by O. graminis and F. annosus. This suggests that more than one enzyme is involved in the breakdown of cellulose and hence supports the observations of Grimes et al. (89) and of Halliwell (95) for cellulose breakdown by enzymes from Myrothecium verrucaria. Both these authors suggest that a rather rare enzyme is involved in the initial breakdown of the longer chains of insoluble cellulose. A second enzyme, more widely distributed than the first, is considered responsible for hydrolysing the linkages of the shorter chains. Halliwell points out that many investigations have been carried out with soluble forms of cellulose with comparatively small molecules, and many so called cellulases of micro-organisms have been found incapable of breaking down insoluble cellulose. He mentions also that many insoluble preparations of cellulose also contain much partially broken down material. This is of particular interest in connection with the results presented here as T. viride and T. asperum have both been reported as cellulose decomposers. It is possible that

TABLE 18. PRODUCTION OF HYDROLYTIC ENZYMES BY TRICHODERMA VIRIDE, TRICHOCLADIUM ASPERUM, OPHIOBOLUS GRAMINIS AND FOMES ANNOSUS.

SUBSTRATE	TYPE OF LINKAGE	CONSTITUENTS	ENZYME	TRICHODERMA VIRIDE	TRICHOCLADIUM ASPERUM	OPHIOBOLUS GRAMINIS	FOMES ANNOSUS
Sucrose	1-alpha-beta	glucose fructose	invertase	Poor	Good	Good	Good
Lactose	1-4-beta	glucose galactose	beta-galactosidase	Good	Uncertain	Good	Good
Melibiose	1-6-alpha	glucose galactose	melibiase	Good	Uncertain	Poor	Good
Maltose	1-4-alpha	glucose glucose	maltase	Fair	Good	Fair	Good
Cellobiose	1-4-beta	glucose glucose	cellobiase	Good	Good	Good	Fair
Inulin	1-2	(fructose)n	inulase	Poor	Good	Poor	Poor
Starch	1-4-alpha and 1-6-alpha	(glucose)n	alpha-amylase beta-amylase 1-6 glucosidase	Good	Good	Good	Good
Pectin	1-4-alpha	(galacturonic acid)n	pectin enzyme complex	Fair	Fair	Fair	Good
Cellulose	1-4-beta	(glucose)n	cellulase	Poor	Poor	Good	Good

the degree of polymerization in the preparation of cellulose powder used here was higher than that used by other investigators (107, 132).

Considering the utilization of carbohydrates as a whole it is difficult to pick out any features which distinguish the saprophytes from the parasites. If anything, the parasites appear to be somewhat superior in the production of hydrolytic enzymes, and perhaps are able to make better use of more of the simple sugars than the saprophytes. For example, more "Goods" appear in Table 18 for the parasites and the totals of the percentage growth on the monosaccharides are greater for the parasites.

The possibility that the formation of adaptive enzymes was required in some cases before the substrates were attacked must not be overlooked. A lag in the onset of enzymic breakdown would appear here as reduced growth; only by determination of growth rates could this possibility be resolved.

It is noted that some growth occurred on all carbohydrates and this was also observed by Lilly and Barnett (125). This could have been due, in addition to utilization of the carbohydrate, to residual glucose transferred with the inoculum and to the fact that asparagine was the nitrogen source and could provide carbon for a limited amount of growth.

DISCUSSION AND CONCLUSIONS.

Although it is not possible to draw an obvious line of distinction between the saprophytes and parasites studied here on the basis of their nutritional requirements certain points stand out and are of interest.

The growth rates of the parasites on solid media were usually intermediate between the two saprophytes. T. viride had a very high growth rate while that of T. asperum was much slower. It should be noted that T. viride in liquid cultures tended to produce a smaller weight of mycelium than the other three fungi. Growth rate alone, therefore, is no criterion of saprophytic ability although it seems reasonable to conclude that under some circumstances it could be of tremendous advantage, e.g. in competing with other fungi.

The relation of temperature to growth was also seen to vary between species; however, at low temperatures O. graminis would probably have some advantage in growth rate over both saprophytes.

The response to pH would indicate that both of the saprophytes are more acid tolerant than the parasites. F. annosus had a rather restricted pH range while that of O. graminis was more extended; under more alkaline conditions total growth of O. graminis was as great as that of either saprophyte.

The observations on nitrogen and vitamin requirements of these fungi were of interest because both parasites showed a preference for organic nitrogen and both required vitamins. Fries (68) has pointed out that vitamin requirements at least among the basidiomycetes appear to occur as much among saprophytes as among parasites, and Schopfer (180) states that

there appear to be adequate supplies of vitamins in the soil. However, this requirement, together with a preference for organic nitrogen, does suggest that the parasites would be favoured more by an environment that provides these substances in abundance, such as the living plant cell.

Given adequate nitrogen and vitamin supplies the parasites were observed to make good growth on rather more carbohydrates than the saprophytes.

It seems entirely reasonable to conclude that from these studies alone combinations of conditions could be selected which would favour each fungus in turn over the others; the conditions of nutrition which appear to favour the parasites are to be found optimally in the living tissue of higher plants.

PART III. INFLUENCE OF INDOLEACETIC ACID AND OTHER GROWTH SUBSTANCES ON THE GROWTH OF TRICHODERMA VIRIDE, TRICHOCLADIUM ASPERUM, OPHIOBOLUS GRAMINIS AND FOMES ANNOSUS.

INTRODUCTION.

For more than 30 years the relationship of indoleacetic acid and other growth substances to the growth and metabolism of higher plants has been a subject of intensive study. It is somewhat surprising to find that this interest has not been paralleled in the case of the fungi, particularly for parasitic fungi where the physiologies of host and invading cells become so intimately associated.

It would be surprising if indoleacetic acid did not have some part to play in the metabolism of fungi. This substance has been shown to have activity for pteridophytes (105, 236), bryophytes (137, 237) and algae (20), as well as for seed plants. Even if fungi are not as closely related to plants as has been commonly assumed (133), a recent report by Woolley (243) suggests that indoleacetic acid or a related substance is of widespread occurrence throughout all living cells.

There have, in fact, been several observations of the production of indoleacetic acid by fungi. Nielsen (144,145) was the first to isolate indoleacetic acid from fungus material. He found that *Rhizopus suinus* and *Absidia ramosa* produced a substance (which he called rhizopin) in their culture media which was active in the *Avena* coleoptile test. The medium consisted of filter paper soaked with a solution of glucose, ammonium tartrate and mineral salts. Boysen Jensen (17) also found

a similar substance to be produced by Aspergillus niger in liquid media containing peptone or haemoglobin. He demonstrated subsequently (18) that simple amino acids, particularly tryptophane, could be substituted for the peptone and haemoglobin and give high yields of this growth substance. Kogl and Kostermans (114) demonstrated the production of a similar substance by yeast and reported that the active substances had a molecular weight similar to that of indoleacetic acid. Thimann (199) actually demonstrated that rhizopin was identical with indoleacetic acid.

These discoveries most certainly had an important influence on the study of growth substances in higher plants, but surprisingly enough inspired no intensive investigations among fungi. This was due probably to several reasons; for example, indoleacetic acid did not appear to produce the effects on fungi that it did on higher plants, whereas other substances were found which did have stimulating effects on fungus growth (115), and also other sources of indoleacetic acid were found.

Nevertheless, there have been occasional reports of the production of indoleacetic acid by fungi up to the present time. Thus Moulton and Link (141), Moulton (140) and Wolf (239) have demonstrated the production of indoleacetic acid by Ustilago zeae. Berducou (7) concluded that the formation of cankers in apple trees invaded by Nectria galligena was due to the secretion of indoelacetic acid by this fungus. Production of indoleacetic acid by Gymnosporangium Juniperis-virginianae has been demonstrated by Wolf (241). Gentile and Klein (77) have demonstrated the production of indoleacetic acid in liquid media by Diplodia natalensis. Pilet (158) found that leaves of Euphorbia parasitized with Uromyces have a higher indoleacetic acid content than

healthy leaves; a similar finding has been reported by Shaw and Hawkins (182) for rusted wheat and mildewed barley leaves.

It is of interest that all these observations have been made on parasitic fungi, several of them because of the morphological changes that have been produced in infected host plants. As the original observations of indoleacetic acid production by fungi were made with saprophytes, there are no grounds for concluding that indoleacetic acid production is a characteristic distinguishing parasitic from saprophytic fungi. It would, however, seem reasonable to conclude that indoleacetic acid production is a fairly general phenomenon in fungi.

Comparatively few investigations have been made of the effect of plant growth substances on fungi. Leonian and Lilly (117) tested the effect of indoleacetic acid on a large number of fungi and failed to obtain any stimulatory response. Fries (68) refers to the reports of Kerl (112) and Defago (51) who claimed considerable increases in the growth of Pyronema confluens and Tilletia tritici, respectively, due to indoleacetic acid. Anker (1) reported an increase in the rate of oxidation of glucose by Saccharomyces cerevisiae in the presence of indoleacetic acid and Berducou (6) claimed indoleacetic acid acted as a mutagen for Nectria galligena. Richards (163) found several growth substances at low concentrations to have minor stimulatory effects on various fungi, high concentrations were inhibitory. Fraser (62) concluded that under some conditions indoleacetic acid stimulated growth of Psalliota hortensis, but was inhibitory under others. Nysterakis (150, 151) reported that Neurospora tetrasperma was not inhibited by high concentrations of indoleacetic acid and that inhibition of Nectria galligena by indoleacetic acid could be overcome by

an unidentified extract of cotton. Gentile and Klein (77) showed that indoleacetic acid inhibited the growth of Diplodia natalensis, but pointed out that the determination of the effects of indoleacetic acid on fungi are complicated by the fact that this substance is produced endogenously, which is not the case in the oat coleoptile. The effect produced by added indoleacetic acid on the growth of a fungus would, therefore, be influenced by the amount already present in the mycelium or secreted into the medium. The latter would in turn vary according to the constituents of the medium - media containing tryptophane would support better indoleacetic acid formation than media containing other sources of nitrogen. They attempted to demonstrate that indoleacetic acid was necessary for the growth of D. natalensis by using 2,4,6-trichlorophenoxyacetic acid as an antimetabolite. Growth was inhibited by the latter substance and this inhibition could be overcome by adding indoleacetic acid.

Petursen (157) found indoleacetic acid to be without effect on the germination of uredospores of Puccinia graminis var tritici except at concentrations of 200 p.p.m. and higher, where it was inhibitory. However, stimulation of germination of uredospores by indoleacetic acid has been reported by Sumere et al. (194).

In addition mention should be made of reports of the value of plant growth substances as systemic fungicides. Thus Crowdy (46) found indolebutyric acid effective in reducing apple canker caused by Nectria galligena, although it had little effect on the organism in culture. Davis and Dimond (48) found several growth substances to reduce disease caused by Fusarium oxysporum f. lycopersici in tomato plants; again little activity was observed in culture. Hemphill and Goodman (98) obtained

increased control of Erwinia amylovora with antibiotic treatments by including indoleacetic acid as well.

A further indication that indoleacetic acid is a natural metabolite of fungi comes from the report of Sequeira and Steeves (181) that an indoleacetic acid oxidase is produced by Omphalia flavida.

To summarize, therefore: fungi have been shown to produce indoleacetic acid both in culture and in living plant hosts; indoleacetic acid has been shown to be physiologically active, inhibiting growth at high concentrations and, possibly under certain conditions, increasing growth at low concentrations; breakdown of indoleacetic acid has been demonstrated for one fungus. Thus it may be concluded that indoleacetic acid is probably important in fungus metabolism and also in the physiology of host-parasite relations.

The experiments to be described below were first initiated on the basis of a report by Waygood et al. (222) that indoleacetic acid stimulated the production of pectin-esterase by Cladosporium herbarum. Unfortunately, to the writer's knowledge, no full account of this work has been published, for such a finding, if confirmed, would be of considerable significance. Attempts to duplicate these results with the fungi studied here were not successful, but additional observations of interest were made.

EFFECT OF INDOLEACETIC ACID AND OTHER PLANT GROWTH SUBSTANCES ON THE GROWTH OF *TRICHODERMA VIRIDE*, *TRICHOCLADIUM ASPERUM*, *OPHIOBOLUS GRAMINIS*, *FOMES ANNOSUS* AND CERTAIN OTHER FUNGI.

MATERIALS AND METHODS.

A determination of the amount of growth under various conditions being the main purpose of the experiments in this section, the materials and methods were mostly the same as in the nutritional studies, Part II. However, certain points require special mention here.

Medium.

The medium used was identical with that employed in the nutritional studies with the exception that in some of the early experiments 1 gram per litre of yeast extract was used instead of the vitamin mixture. Comparison of data indicated that the change from yeast extract to the vitamin mixture did not affect the responses of the fungi to indoleacetic acid.

Inoculum.

Inoculation of solid media was carried out as previously, but the method used for inoculation of liquid cultures differed from that used in most of the experiments described so far. Briefly it was as follows: The culture medium from flask cultures, approximately three weeks old, was removed and each mat then washed aseptically three times with chilled sterile distilled water. The mats were then transferred to a sterile semi-micro monel-metal jar of a Waring blendor assembly, and approximately 20 ml. of chilled ($0^{\circ}C$) sterile 0.25 M sodium chloride added for each

mat used. The mycelium was then disintegrated for 1 minute at 0°C. The resulting suspension served as a uniform viable inoculum. Usually 2 ml. (by pipette) was used to inoculate each culture flask.

The blendor jar was sterilized by first washing in hot detergent and rinsing in hot water, then rising 3 times with cold methyl alcohol and finally rinsing 3 times with cold sterile distilled water.

Indoleacetic acid solutions.

A stock solution of 500×10^{-5} M indoleacetic acid was usually prepared for each experiment. In the early experiment ethyl alcohol was used to aid solution, but because of the possibility that alcohol might be physiologically active, $\frac{N}{20}$KOH was used subsequently. Comparison of the results obtained with the two solvents suggested that ethyl alcohol had no influence on the effect of indoleacetic acid.

Composition of the experimental media.

Solutions of indoleacetic acid were made up at five times the concentration required in the experimental media. The indoleacetic acid solutions were incorporated in the media so that in making up the latter to the final volume they were diluted to the required strength. Final concentrations of 0, 0.01, 0.10, 1.00, 10.00, 50.00 and 100.00×10^{-5} M were used. An equal amount of solvent was added to the control as to the other treatments. The pH was adjusted to 5.5 with $\frac{N}{10}$ hydrochloric acid.

In the experiments involving other growth substances the treatment was similar.

EFFECT OF INDOLEACETIC ACID ON GROWTH WITH PECTIN AS CARBON SOURCE - PRELIMINARY EXPERIMENT.

Waygood et al. (222) reported that growth of Cladosporium herbarum on pectin was stimulated by indoleacetic acid at low concentrations and inhibited at high concentrations. The secretion of pectinesterase was particularly increased by 10^{-5} M indoleacetic acid and inhibited by 10^{-3} M. It was also observed that no such responses occurred with sucrose as carbon source.

A brief investigation was, therefore, carried out to determine if indoleacetic acid could stimulate growth of the fungi under investigation in the present study on pectin.

The methods used were as indicated above. An equivalent amount of pectin was substituted for glucose in the basic medium, and indoleacetic acid was used at 0.00, 0.01, 0.10, 1.00, and 10.00×10^{-5} M.

RESULTS.

Two fungi were used, O. graminis and T. viride (E); the results are given in Table 19.

No stimulation of the growth of either fungus was observed. However, it was noticed that the extent of inhibition of O. graminis at 10×10^{-5} M was much greater than that of T. viride (E) at this concentration. In fact, whereas there was no significant difference in the growth of T. viride (E) with the various concentrations of indoleacetic acid, growth of O. graminis was significantly reduced compared with the control. It was decided, therefore, to investigate the effect of indoleacetic acid further, using additional fungi and a wider range of concentrations.

TABLE 19. EFFECT OF INDOLEACETIC ACID ON THE GROWTH OF *TRICHODERMA VIRIDE* (E) AND *OPHIOBOLUS GRAMINIS* ON A MEDIUM CONTAINING PECTIN AS CARBON SOURCE.

a) *TRICHODERMA VIRIDE* (E)

	INDOLEACETIC ACID CONCENTRATION	MILLIGRAMS DRY MYCELIUM PER FLASK				TOTAL	MEAN
7 days growth	Control	287.4	278.6	271.4	216.7	1054.1	263.5
	0.01 x 10^{-5} M	226.6	278.7	289.8	235.8	1030.9	257.7
	0.10 x 10^{-5} M	256.7	270.8	282.4	270.3	1080.2	270.1
	1.00 x 10^{-5} M	288.2	290.7	215.8	231.5	1026.2	256.6
	10.00 x 10^{-5} M	229.3	217.7	280.9	243.7	971.6	242.9

ANALYSIS OF VARIANCE.

SOURCE OF VARIATION.	D.F.	M.S.	F.	F. at 1%
Treatments	4	405.8	0.47	4.89
Error	15	868.15		
Total	19			

TABLE 19 (CONTINUED).

b) OPHIOBOLUS GRAMINIS.

	INDOLEACETIC ACID CONCENTRATION	MILLIGRAMS DRY MYCELIUM PER FLASK				TOTAL	MEAN
7 days growth	Control	123.5	99.0	100.8	102.8	426.1	106.5
	0.01 x 10^{-5} M	101.5	61.2	121.5	115.5	399.7	99.9
	0.10 x 10^{-5} M	111.5	109.6	113.4	98.8	433.3	108.3
	1.00 x 10^{-5} M	107.4	109.8	109.1	101.5	427.8	107.0
	10.00 x 10^{-5} M	58.7	42.9	43.7	42.7	188.0	47.0

ANALYSIS OF VARIANCE.

SOURCE OF VARIATION.	D.F.	M.S.	F.	F. at 1%	L.S.D. at 1%
Treatments	4	2773.56	14.07	4.89	29.3
Error	15	197.04			
Total	19				

EFFECT OF INDOLEACETIC ACID ON THE GROWTH OF VARIOUS FUNGI IN LIQUID MEDIA.

The experiments to be reported here were carried out as described above with the difference that D-glucose was used as carbon source instead of pectin, and concentrations of 50×10^{-5} M and 100×10^{-5} M indoleacetic acid were added.

RESULTS.

The results obtained for the four fungi primarily considered in these studies are given in Table 20. It can be seen that at none of the concentrations of indoleacetic acid used was the growth of either T. viride or T. asperum significantly reduced from that of the control.

O. graminis did not grow in the presence of indoleacetic acid at a concentration of 50×10^{-5} M, while a concentration of 10×10^{-5} M significantly reduced growth from that of the control. F. annosus showed even greater sensitivity to indoleacetic acid than O. graminis, growth being completely inhibited by a concentration of 10×10^{-5} M and significantly reduced by a concentration of 1×10^{-5} M.

These results would suggest that the two parasitic fungi are inhibited by indoleacetic acid at a much lower concentration than are the saprophytes. It was considered advisable to repeat these experiments and extend them to include other fungi. The results obtained from some of these experiments are summarized in Table 21. The additional species included here were Pyronema sp. obtained from the surface of sterilized soil, Rhizopus sp. from wheat field soil, S.14, a soil saprophyte, from the same soil, Fusarium culmorum and Pythium de Baryanum

TABLE 20. EFFECT OF INDOLEACETIC ACID ON GROWTH OF *TRICHODERMA VIRIDE*, *TRICHOCLADIUM ASPERUM*, *OPHIOBOLUS GRAMINIS* AND *FOMES ANNOSUS*.

a) *TRICHODERMA VIRIDE*.

	INDOLEACETIC ACID CONCENTRATION	MILLIGRAMS DRY MYCELIUM PER FLASK					TOTAL	MEAN
7 days growth	Control	186.0	163.8	165.3	182.4	206.8	904.3	180.9
	50.0×10^{-5} M	174.8	154.1	162.6	171.4	186.1	849.0	169.8
	100.0×10^{-5} M	163.7	187.6	206.4	193.0	190.0	940.7	188.1

ANALYSIS OF VARIANCE.

SOURCE OF VARIATION.	D.F.	M.S.	F.	F. at 1%
Treatments	2	426.4	1.83	6.93
Error	12	232.0		
Total	14			

TABLE 20 (CONTINUED).

b) TRICHOCLADIUM ASPERUM.

	INDOLEACETIC ACID CONCENTRATION	MILLIGRAMS DRY MYCELIUM PER FLASK					TOTAL	MEAN
6 days growth	Control	188.6	193.8	179.0	182.5	174.8	918.7	183.7
	0.01×10^{-5} M	192.5	197.9	216.2	178.4	177.2	962.2	192.4
	0.10×10^{-5} M	195.9	186.4	159.3	184.0	173.2	898.8	179.8
	1.00×10^{-5} M	193.8	183.2	176.1	188.2	158.9	900.2	180.0
	10.00×10^{-5} M	184.0	191.8	192.6	193.2	166.0	927.6	185.5
	50.00×10^{-5} M	202.7	220.2	212.9	189.6	193.4	1018.8	203.8
	100.00×10^{-5} M	200.7	178.3	185.5	175.1	177.4	917.0	183.4

ANALYSIS OF VARIANCE.

SOURCE OF VARIATION.	D.F.	M.S.	F.	F. at 1%
Treatments	6	364.3	2.32	3.53
Error	28	156.9		
Total	34			

TABLE 20 (CONTINUED).

c) OPHIOBOLUS GRAMINIS

	INDOLEACETIC ACID CONCENTRATION	MILLIGRAMS DRY MYCELIUM PER FLASK				TOTAL	MEAN
8 days growth	Control	261.4	236.4	235.9	300.0	1033.7	258.4
	0.01×10^{-5} M	249.1	250.0	263.7	251.0	1013.8	253.5
	0.10×10^{-5} M	243.9	205.4	230.3	215.6	895.2	223.8
	1.00×10^{-5} M	235.8	195.9	253.7	210.4	895.8	224.0
	10.00×10^{-5} M	92.8	171.0	157.4	124.9	546.1	136.5
	50.00×10^{-5} M	No growth					

ANALYSIS OF VARIANCE.

SOURCE OF VARIATION	D.F.	M.S.	F.	F. at 1%	L.S.D. at 1%
Treatments	4	9590.6	15.32	4.89	50.4
Error	15	626.1			
Total	19				

TABLE 20 (CONTINUED).

d) *FOMES ANNOSUS*.

	INDOLEACETIC ACID CONCENTRATION	MILLIGRAMS DRY MYCELIUM PER FLASK			TOTAL	MEAN
7 days growth	Control	73.4	85.6	94.8	253.8	84.6
	0.10×10^{-5} M	79.0	64.6	60.7	204.3	68.1
	1.00×10^{-5} M	53.1	49.7	37.2	140.0	46.6
	10.00×10^{-5} M	No growth				

ANALYSIS OF VARIANCE.

SOURCE OF VARIATION.	D.F.	M.S.	F.	F. at 1%	L.S.D. at 1%
Treatments.	2	1085.3	11.7	10.92	29.1
Error	6	92.75			
Total	8				

TABLE 21. SUMMARY OF EFFECTS OF INDOLEACETIC ACID ON THE GROWTH OF VARIOUS FUNGI, ESTIMATED AS PER CENT GROWTH OF CONTROL.

	INDOLEACETIC ACID CONCENTRATIONS						
	Control (0)	0.01 x 10^{-5} M	0.10 x 10^{-5} M	1.00 x 10^{-5} M	10.00 x 10^{-5} M	50.00 x 10^{-5} M	100.00 x 10^{-5} M
Trichoderma viride	100.0	-	-	-	-	93.9	104.0
Trichoderma viride (E) (1)	100.0	-	-	99.5	101.6	104.1	-
Trichoderma viride (E) (2)	100.0	88.4	88.8	91.8	91.8	88.3	86.9
Trichoderma viride (E) (3)	100.0	101.2	93.7	104.5	99.8	111.5	-
Trichoderma viride (E) (4)	100.0	97.8	102.5	97.4	92.2	-	-
Trichocladium asperum (1)	100.0	104.7	97.8	98.0	101.0	110.9	99.8
Trichocladium asperum (2)	100.0	103.3	98.4	95.4	99.1	109.0	72.9
S.14	100.0	95.9	92.9	93.2	100.2	88.9	72.5
Pyronema sp.	100.0	100.5	100.2	99.9	102.8	110.1	-
Rhizopus sp.	100.0	-	-	95.9	100.8	101.4	-
Fusarium culmorum	100.0	-	-	101.8	98.5	104.2	-
Ophiobolus graminis (1)	100.0	-	-	69.7	24.6	0	-
Ophiobolus graminis (2)	100.0	98.7	90.6	78.4	29.2	0	0
Ophiobolus graminis (3) (Pectin)	100.0	98.1	86.6	86.7	52.8	0	0
Pythium de Baryanum	100.0	-	-	98.0	97.5	24.9	-
Botrytis sp.	100.0	-	-	107.0	111.0	31.6	-
Fomes annosus	100.0	80.5	55.1	0	0	0	0

from the laboratory culture collection, and Botrytis sp. isolated from a diseased onion bulb.

The data in Table 21 confirmed the previous results, indicating that under the conditions of the experiments the saprophytic fungi were quite tolerant to concentrations of 100 x 10^{-5} M indoleacetic acid while for the most part the parasites were inhibited by much lower concentrations.

INVESTIGATION OF APPARENT INCREASES IN DRY WEIGHT OF MYCELIUM DUE TO INDOLEACETIC ACID.

From Table 21 it would appear that various concentrations of indoleacetic acid supported better growth than did the controls. Except for Botrytis sp. this appeared to be true only for the saprophytes. However, when such data were analysed statistically the increases in growth were found to fall short of the requirements for significance, except in one case. The pertinent data have been taken from the various experiments and summarized in Table 22.

The increase in growth of T. asperum at 50 x 10^{-5} M indoleacetic acid was significant in one experiment and almost significant in a second. As there have been occasional reports in the literature of stimulation of fungus growth by indoleacetic acid it was decided to carry out a further experiment with T. viride and T. asperum using a range of concentrations from 20 x 10^{-5} M to 100 x 10^{-5} M. It was considered possible that an optimum indoleacetic acid concentration might lie between the concentrations previously used, and hence the stimulation previously observed would be less than the optimum.

The procedure followed was the same as was used previously and the

TABLE 22. INCREASE IN GROWTH OF VARIOUS FUNGI IN THE PRESENCE OF INDOLEACETIC ACID.

INDOLEACETIC ACID CONCENTRATION	MILLIGRAMS DRY MYCELIUM						
	T. viride	*T. viride* (E)	*T. viride* (E)	*T. asperum*	*T. asperum*	*Pyronema* SP.	*Botrytis* SP.
Control	180.9	130.4	285.8	183.7	250.7	225.2	106.2
10×10^{-5} M	-	-	-	-	-	-	117.9
50×10^{-5} M	-	136.0	318.7	203.8	273.3	247.9	-
100×10^{-5} M	188.1	-	-	-	-	-	-
Difference	7.2	5.6	32.9	20.1	22.6	22.7	11.4
L.S.D. at 1%	29.1	10.1	34.4	21.5	17.2	34.8	27.9

concentrations of indoleacetic acid used are indicated in Table 23.

RESULTS.

The results are given in Table 23. No significant differences were found between the amounts of growth at the various concentrations for either fungus.

Thus it is concluded that at the concentrations used in these experiments, indoleacetic acid produced no consistent increase in dry weight of mycelium of the fungi studied. The occasional stimulation observed does, however, suggest that there may be some conditions under which indoleacetic acid can cause an increase in growth, but that these apparently were not the conditions operating in these experiments.

It should be pointed out that in addition to being influenced by an endogenous production of indoleacetic acid (77) the experiments carried out here and those of other investigators differ from Avena coleoptile experiments in that in the latter an increase in growth in length of a part of an organ is measured. Observations of increase in growth of fungus material under the influence of indoleacetic acid should be expected to compare with the type of response found in higher plants only if the fungus material is placed in a comparable physiological condition and data of the same type are recorded. This does not appear to have been appreciated by those investigators who have attempted to obtain growth responses in fungi. Their experiments and those recorded here do not distinguish between growth as a response to nutrition and growth as a hormone response. Thus although the effect of a growth substance may be very small when compared to the overall increase in

TABLE 23. GROWTH OF TRICHODERMA VIRIDE AND TRICHOCLADIUM ASPERUM IN VARIOUS CONCENTRATIONS OF INDOLEACETIC ACID.

a) TRICHODERMA VIRIDE.

	INDOLEACETIC ACID CONCENTRATION	MILLIGRAMS DRY MYCELIUM PER FLASK					TOTAL	MEAN
7 days growth	Control	175.0	160.0	161.0	163.0	136.2	805.1	161.0
	20 x 20^{-5} M	147.5	134.5	174.1	181.2	177.3	814.6	162.9
	40 x 30^{-5} M	175.7	128.2	141.1	129.0	159.5	733.5	146.7
	50 x 40^{-5} M	178.1	167.0	169.3	146.4	145.8	806.6	161.3
	60 x 50^{-5} M	140.8	177.4	180.0	173.9	173.3	845.4	169.1
	80 x 60^{-5} M	145.3	140.4	132.7	194.2	191.4	804.0	160.8
	100 x 70^{-5} M	184.0	173.1	131.0	138.8	159.0	785.9	157.2

ANALYSIS OF VARIANCE.

SOURCE OF VARIATION.	D.F.	M.S.	F.	F. at 1%
Treatments	6	232.6	0.56	3.53
Error	28	416.6		
Total	34			

TABLE 23 (CONTINUED).

b) TRICHOCLADIUM ASPERUM

	INDOLEACETIC ACID CONCENTRATION	MILLIGRAMS DRY MYCELIUM PER FLASK					TOTAL	MEAN
6 days growth	Control	185.6	165.7	158.3	158.8	177.5	845.9	169.2
	20 x 20^{-5} M	162.1	162.8	200.7	182.3	170.8	878.7	175.7
	40 x 30^{-5} M	176.8	155.1	162.3	162.4	176.9	833.5	166.7
	50 x 40^{-5} M	176.1	177.4	193.7	160.9	174.3	882.4	176.5
	60 x 50^{-5} M	164.5	194.7	181.8	180.1	165.4	886.5	177.3
	80 x 60^{-5} M	164.6	168.6	174.9	177.3	178.1	863.5	172.7
	100 x 70^{-5} M	192.7	187.6	185.6	153.6	183.0	902.5	180.5

ANALYSIS OF VARIANCE.

SOURCE OF VARIATION	D.F.	M.S.	F.	F. at 1%
Treatments	6	117.1	0.77	3.53
Error	28	152.6		
Total	34			

weight of a growing organism, its effect in increasing the size of a non-growing part may be considerable. To determine whether indoleacetic acid is effective as a growth substance for fungi, experiments must be designed to test the effects of this substance under conditions where growth is not occurring due to the influence of other factors. It is of interest to note in this connection that, in spite of the marked effect on enlargement of parts of higher plants, application of indoleacetic acid to whole plants has generally not been found to cause an increase in total growth. The reverse is in fact usually the case (120).

EFFECT OF INDOLEACETIC ACID ON THE GROWTH OF VARIOUS FUNGI ON A SOLID MEDIUM.

Fraser (62) reported that in his experiments the effects of indoleacetic acid on the growth of *Psalliota hortensis* were different on solid than on liquid media. It was considered advisable, therefore, to test the generality of the observations made above for the growth of fungi on solid media containing indoleacetic acid.

MATERIALS AND METHODS.

The medium consisted of the basic medium solidified with agar; 15 ml. of medium were used per Petri dish and three dishes were used for each treatment. Two concentrations of indoleacetic acid were used - 10×10^{-5} M and 100×10^{-5} M. Inoculation was carried out in the usual way with 5 m.m. plugs cut from the edges of colonies growing on the basic medium.

Several additional fungi were used here, including several unidentified isolates obtained from the same soil sample as *T. viride* and *T. asperum*,

and these are indicated by the letter S. Other species were from the laboratory culture collection except for a second isolate of F. annosus (C.297) obtained from the Forest Pathology Laboratory, Calgary.

RESULTS.

Growth was measured as the diameter of the fungus colony in millimetres, but as in general the results support those obtained for liquid cultures they are recorded in summary only. In Table 24 growth in the presence of various concentrations of indoleacetic acid is given as percentages of that of the control.

Although the parasitic fungi were not inhibited by such low concentrations of indoleacetic acid as they were in the liquid cultures, and considerably more variation in response occurred among the saprophytes, the same trend was again apparent, indicating that the differential effect of indoleacetic acid on the growth of the parasitic and saprophytic fungi studied in liquid culture was not merely a factor of the experimental conditions used.

INFLUENCE OF ADDITIONAL COMPOUNDS ON THE INHIBITORY EFFECT PRODUCED BY INDOLEACETIC ACID.

A wide variety of substances have been reported as influencing the response of higher plants to indoleacetic acid. An experiment was designed to see if additional compounds could affect the response of fungi to indoleacetic acid.

TABLE 24. EFFECT OF INDOLEACETIC ACID ON THE GROWTH OF VARIOUS FUNGI ON A SOLID MEDIUM ESTIMATED AS PER CENT GROWTH OF CONTROL.

	INDOLEACETIC ACID CONCENTRATION		
	Control	10×10^{-5} M	100×10^{-5} M
Trichoderma viride	100.0	98.9	83.7
Trichoderma viride (E)	100.0	100.5	92.2
Trichocladium asperum	100.0	94.6	96.5
Pyronema sp.	100.0	100.0	100.0
Gliocladium sp.	100.0	100.0	85.4
Rhizopus sp.	100.0	93.3	73.2
S.5	100.0	89.4	91.5
S.6	100.0	100.0	91.5
S.7	100.0	100.6	73.7
S.8	100.0	89.9	78.5
S.9	100.0	99.1	102.7
S.10	100.0	87.1	82.4
S.13	100.0	98.2	101.8
S.14	100.0	100.0	100.0
S.17	100.0	100.0	90.3
Botrytis sp.	100.0	92.7	59.9
Helminthosporium sativum	100.0	81.9	68.6
Ophiobolus graminis	100.0	94.6	32.9
Fomes annosus (C.296)	100.0	78.8	0
Fomes annosus (C.297)	100.0	27.1	0

MATERIALS AND METHODS.

The additional materials used were as follows:

a) Biotin at 100 micrograms per litre; Thimann (200) has suggested that this vitamin might act as an anti-auxin.

b) Adenine at 1 milligram per litre; this substance was reported to overcome indoleacetic acid inhibition of bud formation in tissue culture (136, 184).

c) Adenosine at 1 milligram per litre; also reported effective in the same way as adenine.

d) 2,4,6-Trichlorophenoxyacetic acid at 5×10^{-3} M; reported by Gentile and Klein (77) to act as an antimetabolite of indoleacetic acid in *Diplodia natalensis*.

e) Maleic hydrazide at 5×10^{-3} M. This substance has been considered to act as an anti-auxin by some authors (121).

f) Boron has been shown to be essential in the rooting of cuttings (120), a process also influenced by indoleacetic acid. Boric acid was used here to give a concentration of boron of 1 milligram per litre.

g) Calcium has been shown by Burstrom (36) to prevent adaptation of roots to indoleacetic acid. Calcium chloride was used at 5 grams per litre.

h) 2,4-Dichlorophenol was found by Goldacre et al. (82) to be the most active of a group of substances activating indoleacetic acid oxidase in peas. A concentration of 10^{-4} M was chosen.

These materials were each incorporated into 15% water agar and 15 ml. portions of the resulting mixture were allowed to solidify in Petri dishes.

The four fungi, T. viride, T. asperum, O. graminis and F. annosus, were used in this experiment.

In making up the growth medium indoleacetic acid was used at a concentration of 250 x 10^{-5} M. The medium alone, consisting of the basic medium with agar added, was used as a control.

For each fungus 2 ml. of a mycelium homogenate, prepared as indicated previously, was pipetted into the bottom of a Petri dish. This was followed by 15 ml. of the molten growth medium cooled to 40°C. which was immediately mixed with the homogenate and allowed to solidify. Plugs were then cut from each of the water agar preparations listed above and transferred to the surface of the inoculated growth medium. The plugs were placed close to the edge of the plate and at points equidistant one from another. Three replicates were prepared per fungus.

It was hoped that the indoleacetic acid would inhibit growth of the parasites and, possibly, partially the saprophytes except in the vicinity of plugs from which there was diffusion of a substance active in overcoming the inhibitory effect of the indoleacetic acid.

The results, however, did not bear out this hypothesis in the way that was intended, and will not be discussed at length. Growth of O. graminis and F. annosus was inhibited as anticipated; growth of T. viride and T. asperum was not markedly inhibited. None of the added substances, under the conditions of this experiment, was able to overcome the inhibition of O. graminis and F. annosus by indoleacetic acid. In the case of T. viride and T. asperum, however, the growth in the vicinity of the 2,4-dichlorophenol was less in comparison to the growth in the rest of the plate in the presence of indoleacetic acid than

in the control.

In other words, from this purely visual inspection, it appeared that the interaction of indoleacetic acid and 2,4-dichlorophenol was inhibitive for T. viride and T. asperum. It was obvious that a more complete analysis would be required before definite conclusions could be drawn.

EFFECT OF 2,4-DICHLOROPHENOL ON THE RESPONSE OF TRICHODERMA VIRIDE, TRICHOCLADIUM ASPERUM, OPHIOBOLUS GRAMINIS AND FOMES ANNOSUS TO INDOLEACETIC ACID.

MATERIALS AND METHODS.

The experiment was carried out in the basic medium solidified with agar. In this the following treatments were incorporated:

1. Control.
2. Indoleacetic acid 100 x 10^{-5} M.
3. Indoleacetic acid 100 x 10^{-5} M plus 2,4-dichlorophenol 10^{-4} M.
4. 2,4-Dichlorophenol 10^{-4} M.

The usual procedure was followed; inoculation was carried out with plugs from agar media, four plates were prepared per treatment. Growth was measured in millimetres.

RESULTS.

The results are recorded in Table 25. They are not recorded in full, replicate plates being very similar. In general the expectations from the previous experiment are supported. In the case of T. asperum,

2,4-dichlorophenol does not appear to have increased the sensitivity of this organism to indoleacetic acid, the combination causing a similar amount of inhibition as the 2,4-dichlorophenol alone. _T. viride_, however, is more strongly inhibited by the combination than by 2,4-dichlorophenol and the effect would not appear to be simply additive. Both _O. graminis_ and _F. annosus_ were completely inhibited by the combination but the response to the two substances separately was such as to prevent any further analysis.

TABLE 25. EFFECT OF 2,4-DICHLOROPHENOL ON RESPONSE OF _TRICHODERMA VIRIDE_, _TRICHOCLADIUM ASPERUM_, _OPHIOBOLUS GRAMINIS_ AND _FOMES ANNOSUS_ TO INDOLEACETIC ACID.

	MEAN GROWTH AS PER CENT OF CONTROL			
	T. VIRIDE	_T. ASPERUM_	_O. GRAMINIS_	_F. ANNOSUS_
Control	100.0	100.0	100.0	100.0
Indoleacetic acid 100×10^{-5} M	96.9	96.2	46.2	0
Indoleacetic acid 100×10^{-5} M and 2,4-Dichlorophenol 10^{-4} M	65.8	75.0	0	0
2,4-Dichlorophenol 10^{-4} M	79.4	76.0	36.3	70.6

It would have been of interest to repeat the experiment using low concentrations of indoleacetic acid, not inhibitory to the parasites, but

the possible significance of these observations was not realized until subsequently and the investigation was not pursued further.

EFFECT OF INDOLEBUTYRIC ACID AND 2,4-DICHLOROPHENOXYACETIC ACID ON THE GROWTH OF *TRICHODERMA VIRIDE*, *TRICHOCLADIUM ASPERUM*, *OPHIOBOLUS GRAMINIS* AND *FOMES ANNOSUS*.

Indoleacetic acid having been found to have the effects reported above on the growth of fungi, study was made of the effects of two other substances known to act as growth substance in higher plants.

MATERIALS AND METHODS.

Indolebutyric acid and 2,4-dichlorophenoxyacetic acid were incorporated in solid media in the same manner previously described for indoleacetic acid, at the concentrations indicated in Table 26.

RESULTS.

Growth was measured as the diameter of the fungus colony and growth rates were actually determined, but for the sake of brevity only total growth is recorded in Table 26, estimated as per cent of the control.

It appears from the results that 2,4-dichlorophenoxyacetic acid at the concentrations used does not appreciably inhibit the growth of any of the fungi. Indolebutyric acid does have some inhibitory action, particularly for the parasites, although the inhibition is not as strong as in the case of indoleacetic acid.

TABLE 26. EFFECT OF INDOLEBUTYRIC ACID AND 2,4-DICHLOROPHENOXYACETIC ACID ON THE GROWTH OF *TRICHODERMA VIRIDE*, *TRICHOCLADIUM ASPERUM*, *OPHIOBOLUS GRAMINIS* AND *FOMES ANNOSUS*.

INDOLEBUTYRIC ACID CONCENTRATION	GROWTH AS PER CENT OF CONTROL.			
	T. VIRIDE	*T. ASPERUM*	*O. GRAMINIS*	*F. ANNOSUS*
Control	100.0	100.0	100.0	100.0
1×10^{-5} M	100.0	91.9	96.3	103.9
10×10^{-5} M	106.2	104.6	93.9	95.0
100×10^{-5} M	106.2	70.1	34.3	70.7
250×10^{-5} M	92.7	64.4	0	37.9
2,4-DICHLOROPHENOXY-ACETIC ACID CONCENTRATION				
Control	100.0	100.0	100.0	100.0
1×10^{-5} M	100.0	100.9	91.2	99.4
10×10^{-5} M	99.5	97.4	93.3	98.8
100×10^{-5} M	101.1	98.6	90.6	96.4
250×10^{-5} M	99.5	96.9	75.5	98.2

INVESTIGATION OF THE INHIBITORY EFFECT OF INDOLEACETIC ACID ON FUNGI.

To explain the difference in the response of the parasitic and saprophytic fungi to indoleacetic acid three suggestions may be made.

1. Indoleacetic acid synthesis may occur in the parasitic fungi to a greater degree than in the saprophytes, resulting in an increased concentration of indoleacetic acid in the medium of the parasites, there being no true difference in susceptibility.

2. The saprophytes may not absorb indoleacetic acid from the medium, the parasites may.

3. Differences may occur in the ability of the two groups to destroy indoleacetic acid enzymatically. The saprophytes would be expected to have a greater ability to do this than the parasites.

With regard to 1, the following observations make it unlikely.

Indoleacetic acid inhibited the growth of O. graminis and F. annosus completely at a concentration usually of 10×10^{-5} M for the former and 1×10^{-5} M for the latter. The concentration required for complete inhibition of T. viride and T. asperum was not determined, but was certainly in excess of 150×10^{-5} M in liquid media, and growth occurred at 500×10^{-5} M on solid media. Therefore if it is assumed that as high a concentration as 50×10^{-5} M indoleacetic acid is required to inhibit O. graminis and F. annosus and that T. viride and T. asperum are inhibited by as low a concentration as 150×10^{-5} M, which they are not, then if the difference in inhibition of the two groups is to be explained by a greater production of indoleacetic acid by the parasites, the latter

must be able to develop a concentration of at least 100 x 10^{-5} M indoleacetic acid in their culture medium. That this was not the case was demonstrated by the use of Salkowski reagent which indicated that the concentration, if any, of indoleacetic acid in the culture media which had supported the growth of these fungi was below that necessary to give a colour reaction, which is in the neighbourhood of 1 x 10^{-5} M. It should be pointed out that a concentration of 100 x 10^{-5} M indoleacetic acid lies above the usable part of the colour range of the Salkowski test.

The question of differential absorption was not resolved, but the results of the experiments to be described below in support of the third hypothesis make it unlikely that this is the main factor operating.

The third possibility was, therefore, studied experimentally.

METHOD OF DETERMINATION OF INDOLEACETIC ACID.

Indoleacetic acid concentrations were determined colorimetrically by a modification (84) of the method described by Salkowski (178). The reagent consisted of 1 ml. 0.5 M ferric chloride added to 50 ml. 35% perchloric acid. 2 ml. of this reagent were added to 1 ml. of sample to be tested and the optical density of the mixture was determined after 25 minutes at a wavelength of 530 millimicrons, using a Bausch and Lomb colorimeter ('Spectronic 20').

A standard curve of the optical density for a range of concentrations of indoleacetic acid in the basic medium was prepared and is reproduced as Figure 10.

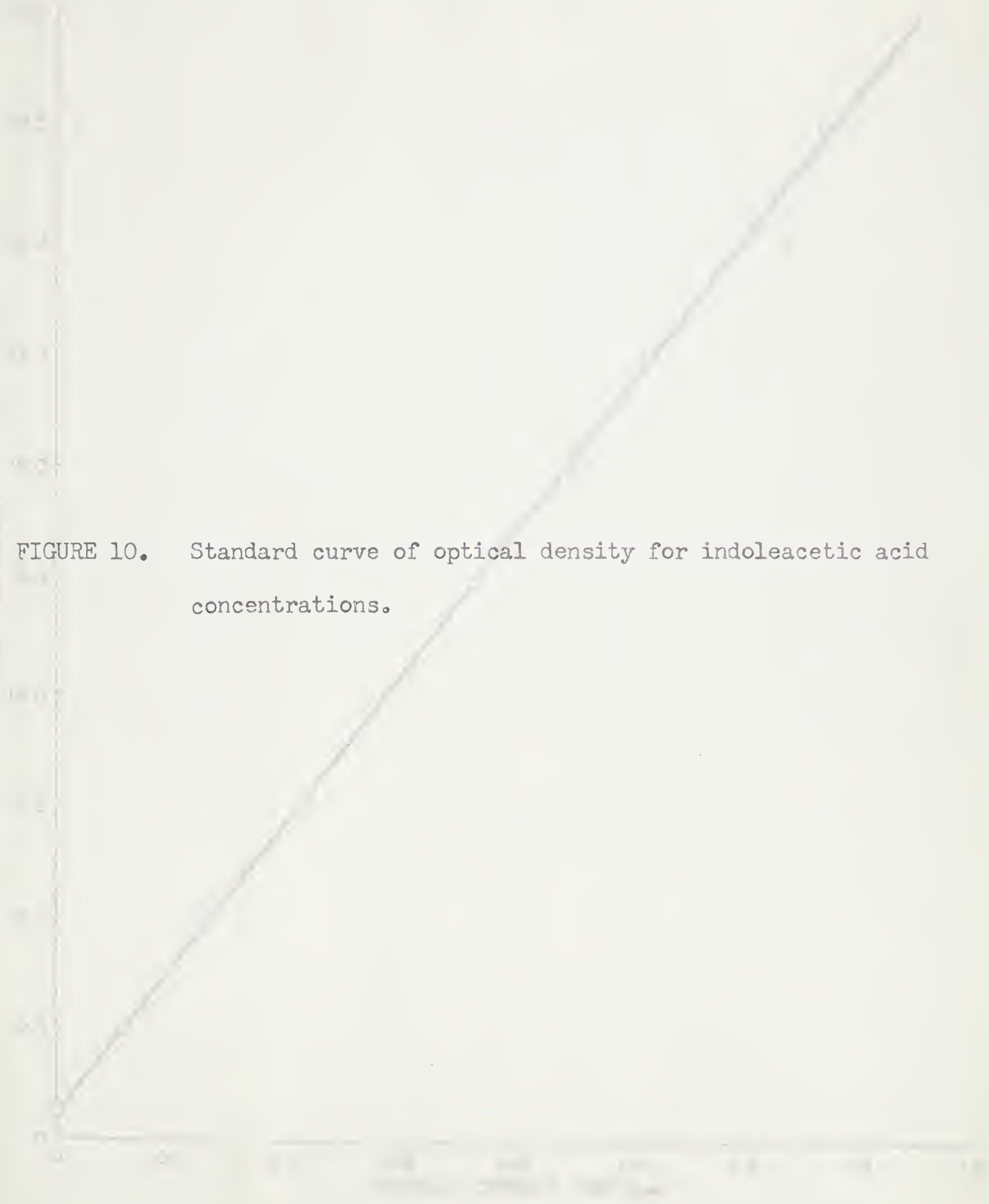
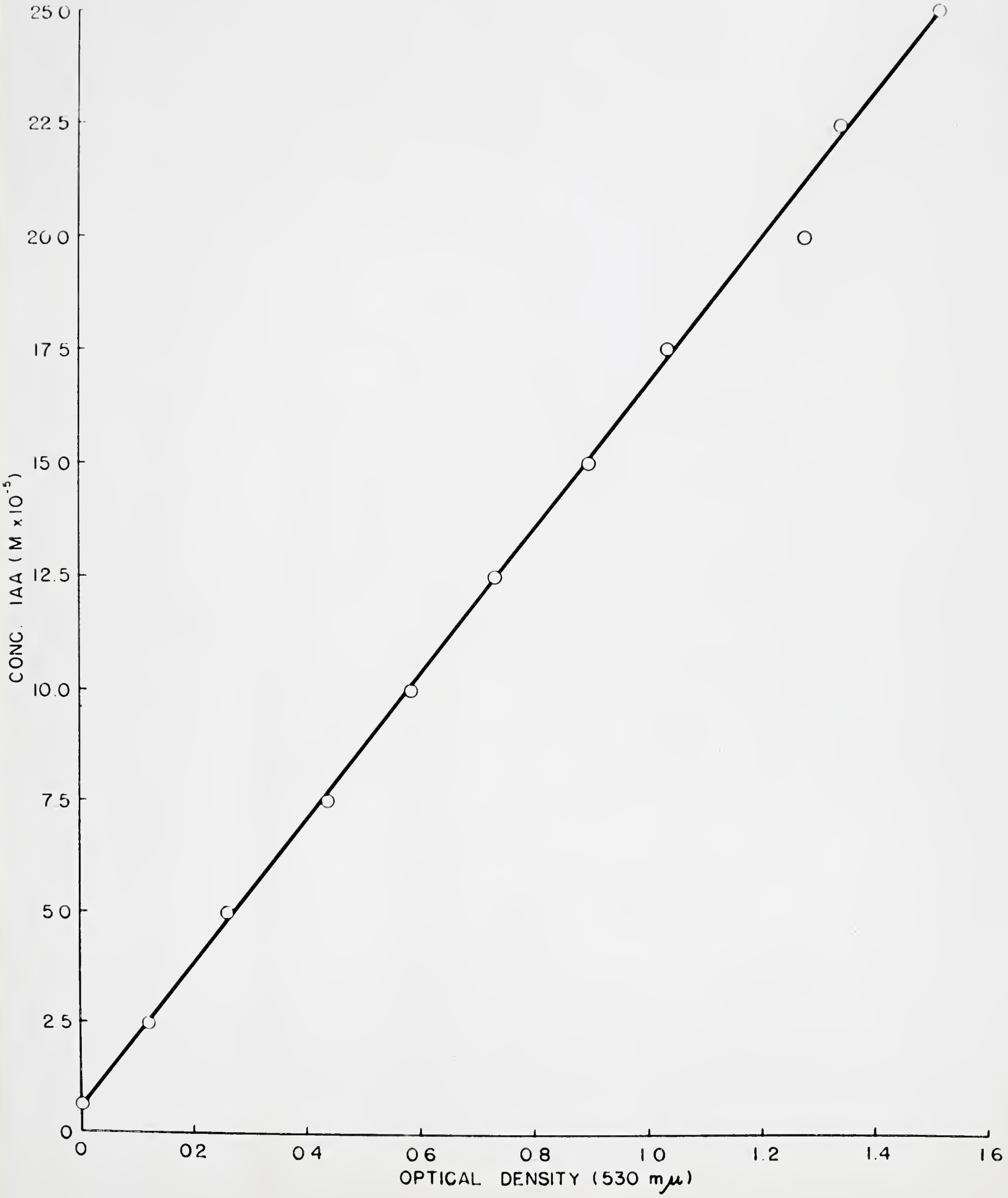


FIGURE 10. Standard curve of optical density for indoleacetic acid concentrations.


25.0
22.5
20.0
17.5
15.0
12.5
10.0
7.5
5.0
2.5
0
CONC. IAA (M x 10^{-5})
0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
OPTICAL DENSITY (530 mμ)

BREAKDOWN OF INDOLEACETIC ACID BY MYCELIAL MATS.

MATERIALS AND METHODS.

The culture medium from beneath fungus mats in flask was removed and the under surface of the mat was washed by adding and removing successively three aliquots of sterile distilled water. One series of mats was then autoclaved for the purpose of providing a killed control. However, the effect on the mycelium was such that it could not be used in this way and so was omitted from the experiment. To a second series of mats (3 for each fungus) a 25 ml. aliquot of the sterilized basic medium containing indoleacetic acid at a concentration of 25×10^{-5} M was added. To a third series of mats the basic medium without indoleacetic acid was added. 1 ml. aliquots of medium were removed at intervals and the concentrations of indoleacetic acid determined as described. The mats were then dried and weighed.

RESULTS.

The results are given in Table 27, where the breakdown of indoleacetic acid has been determined from the standard curve. It can be seen that breakdown of indoleacetic acid occurred to a greater extent with the mats of *F. annosus* and *O. graminis* than with *T. viride* or *T. asperum*. This was somewhat surprising as it had been anticipated that activity, if any, would be found among the saprophytes, explaining their greater resistance to indoleacetic acid.

TABLE 27. BREAKDOWN OF INDOLEACETIC ACID BY MATS OF *TRICHODERMA VIRIDE*, *TRICHOCLADIUM ASPERUM*, *OPHIOBOLUS GRAMINIS* AND *FOMES ANNOSUS*.

	MICROMOLES INDOLEACETIC ACID BROKEN DOWN PER MILLIGRAM OF DRY MYCELIUM		
	35 minutes	120 minutes	450 minutes
T. viride	0.034	0.310	0.315
T. asperum	0.008	0.248	0.238
O. graminis	0.130	0.354	0.455
F. annosus	0.076	0.546	0.730

BREAKDOWN OF INDOLEACETIC ACID BY MYCELIAL HOMOGENATES.

It was of particular interest to determine whether indoleacetic acid breakdown could be brought about by homogenates of the type used for inoculation purposes in experiments where inhibition of *O. graminis* and *F. annosus* had been demonstrated. Satisfactory demonstration of activity under the conditions used was obtained only for *F. annosus* but this confirmed the previous observations for mats, that parasitic fungi have the ability to break down indoleacetic acid.

MATERIALS AND METHODS.

The homogenate was prepared as already indicated, and the dry weight of a sample determined.

The reaction mixtures and controls were:

1. Basic medium with indoleacetic acid 20 x 10^{-5} M 40 ml., homogenate 10 ml.

2. Basic medium with indoleacetic acid 20×10^{-5} M 40 ml., boiled homogenate 10 ml.
3. Basic medium without indoleacetic acid 40 ml., homogenate 10 ml.
4. Basic medium with indoleacetic acid 20×10^{-5} M 40 ml., distilled water 10 ml.

The experiment was carried out in 200 ml. flasks in triplicate. The flasks were shaken at room temperature.

At 45 minute intervals 5 ml. samples of the mixtures were removed and placed in test tubes in a water bath at 100°C for 10 minutes. The samples were then centrifuged at 5000 r.p.m. for 15 minutes and the concentration of indoleacetic acid in the supernatant determined colorimetrically.

RESULTS.

The mean values obtained for the destruction of indoleacetic acid by the homogenate are given in Table 28.

These values were converted to the amount of breakdown per milligram dry homogenate and are recorded in Table 29 and Figure 11.

It can be seen from Table 28 that the homogenate of F. annosus was highly active in destroying indoleacetic acid and as might be expected higher activity was found per milligram than in the case of the mats (Table 29). No further studies on the enzymes involved or on the presence of similar enzymes in homogenates of the other fungi were carried out. The observations made here are considered sufficient to indicate that breakdown of indoleacetic acid occurred when a homogenate of

F. annosus was added to the basic medium containing indoleacetic acid. It should be emphasized that the conditions used were comparable to those used in the inoculation of flasks where inhibition of growth by indoleacetic acid was recorded.

TABLE 28. DESTRUCTION OF INDOLEACETIC ACID BY A HOMOGENATE OF FOMES ANNOSUS.

	INDOLEACETIC ACID DESTRUCTION IN MICROMOLES					
	TIME (mins.)					
	45	90	135	180	225	270
Indoleacetic acid and homogenate	11.0	52.0	76.0	99.5	112.0	118.2
Indoleacetic acid and boiled homogenate	- 1.63	- 1.38	- 0.50	- 0.50	0.50	0.50
Indoleacetic acid	- 2.1	0	- 2.1	- 0.125	0.50	- 0.125

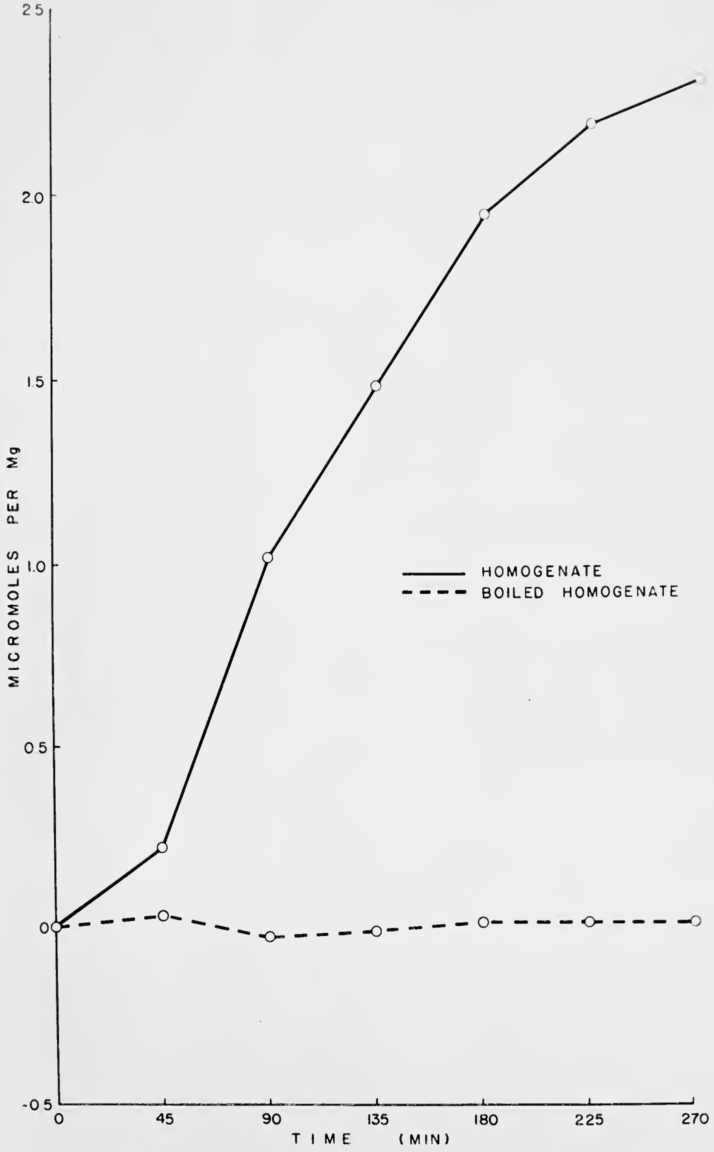
EFFECT OF HOMOGENATES OF OPHIOBOLUS GRAMINIS AND FOMES ANNOSUS ON THE RESPONSE OF TRICHODERMA VIRIDE AND TRICHOCLADIUM ASPERUM TO INDOLEACETIC ACID.

The observations reported above suggest that if differences occur in the ability of the two saprophytes and the two parasites to absorb indoleacetic acid from the medium this is not the major cause of the differences in their tolerance to indoleacetic acid. The main cause of this difference would appear to lie in the ability of the parasites to

FIGURE 11. Breakdown of indoleacetic acid by a crude homogenate of _Fomes_ _annosus_, activity determined colorimetrically and indicated as micromoles indoleacetic acid broken down per milligram dry homogenate. The data are taken from Table 29 below.

TABLE 29. BREAKDOWN OF INDOLEACETIC ACID BY A CRUDE HOMOGENATE OF _FOMES_ _ANNOSUS_.

	INDOLEACETIC ACID BROKEN DOWN AS MICROMOLES PER MILLIGRAM DRY HOMOGENATE.					
			TIME (mins.)			
	45	90	135	180	225	270
Indoleacetic acid and homogenate	0.216	1.020	1.490	1.951	2.196	2.318
Indoleacetic acid and boiled homogenate	0.032	- 0.027	- 0.010	0.010	0.010	0.010


2.5
2.0
1.5
1.0
0.5
0
-0.5
MICROMOLES PER Mg
0
45
90
135
180
225
270
TIME (MIN)
HOMOGENATE
BOILED HOMOGENATE

cause some breakdown of the indoleacetic acid molecule such that it can no longer be detected by the colour reaction used. If this is the case, and if the saprophytes differ in that they do not break down the indoleacetic acid molecule, then it might be expected that the products resulting from the activity of O. graminis and F. annosus would inhibit growth of T. viride and T. asperum where indoleacetic acid would not.

To test this hypothesis the following experiments were performed.

MATERIALS AND METHODS. (a)

Homogenates of all four fungi were prepared. To each of a series of flasks containing 20 ml. of the basic medium with various concentrations of indoleacetic acid 2 ml. of a homogenate of either T. viride or T. asperum was added. The flasks were then divided into three groups; to the flasks of one group 2 ml. of O. graminis homogenate was added, to those of the second group 2 ml. of F. annosus homogenate was added, and the third was left as a control. The layout of the various treatments was as follows:

INDOLEACETIC ACID	HOMOGENATE ADDED		
CONCENTRATION	No addition	O. graminis	F. annosus
Control	1	7	13
0.1×10^{-5} M	2	8	14
1.0×10^{-5} M	3	9	15
10.0×10^{-5} M	4	10	16
50.0×10^{-5} M	5	11	17
100.0×10^{-5} M	6	12	18

The flasks were then incubated at room temperature for seven days, after which observations were made and the dry weight of mycelium produced in certain treatments recorded.

RESULTS.

From a visual inspection no differences were detectable between the various treatments in the case of *T. viride*, but considerable differences were apparent in the case of *T.asperum*. The mycelium from flasks in which these differences occurred, together with that from the controls was harvested and the dry weight of the mycelium determined. These results are given in Table 30.

It can be seen that the homogenates were without effect on the growth of *T. asperum* in the control, and indoleacetic acid had a comparatively small effect on the growth of *T. asperum* in the absence of an added homogenate. The combination of *F. annosus* homogenate and indoleacetic acid at 50 and 100 x 10^{-5} M produced a very marked reduction in growth and likewise the *O. graminis* homogenate produced a considerable decrease in the amount of growth at the higher concentrations. This is evidence in support of the hypothesis that the breakdown products of indoleacetic acid produced by *O. graminis* and *F. annosus* are inhibitory to growth. A check on the concentration of indoleacetic acid remaining in the medium after 7 days growth also supported this conclusion, inhibition of growth being correlated with greatest indoleacetic acid breakdown; the values are given in Table 31.

TABLE 30. EFFECT OF HOMOGENATES OF *OPHIOBOLUS GRAMINIS* AND *FOMES ANNOSUS* ON RESPONSE OF *TRICHOCLADIUM ASPERUM* TO INDOLEACETIC ACID.

		MILLIGRAMS DRY MYCELIUM		
			HOMOGENATE ADDED	
		NIL	*O. GRAMINIS*	*F. ANNOSUS*
Control		153.7	173.3	156.9
		157.5	137.5	148.2
		161.4	178.8	177.5
	Total	472.6	489.6	482.6
Indoleacetic acid 50×10^{-5} M		149.9	145.7	107.3
		174.7	185.2	82.0
		153.1	143.6	79.7
	Total	477.7	474.5	269.0
Indoleacetic acid 100×10^{-5} M		108.9	100.3	52.4
		171.2	97.0	76.9
		131.9	96.2	74.0
	Total	412.0	293.5	203.3

TABLE 31. EFFECT OF HOMOGENATE OF FOMES ANNOSUS ON THE RESIDUAL CONCENTRATION OF INDOLEACETIC ACID IN MEDIA AFTER SEVEN DAYS GROWTH OF TRICHOCLADIUM ASPERUM.

INDOLEACETIC ACID CONCENTRATION	CONTROL	PLUS HOMOGENATE
100×10^{-5} M	30×10^{-5} M	11.5×10^{-5} M
50×10^{-5} M	13.25×10^{-5} M	3.5×10^{-5} M

Additional support was obtained in a further experiment.

MATERIALS AND METHODS. (b)

In general the methods were the same as in the previous experiment. However, only one concentration of indoleacetic acid was used, and in addition to the crude homogenates of O. graminis and F. annosus, cell free extracts and boiled controls were also used. The various treatments are indicated in Table 32. The cell free extract was obtained as the supernatant after centrifuging the crude homogenate for 30 minutes at 5000 r.p.m. The homogenates and supernatants were boiled by autoclaving under standard conditions. Three flasks were used per treatment. The mycelium was harvested after 7 days growth.

RESULTS.

For T. viride there were again insufficient differences between the treatments for significance. In the case of T. asperum, F. annosus

TABLE 32. EFFECT OF HOMOGENATES AND CELL FREE EXTRACTS OF OPHIOBOLUS GRAMINIS AND FOMES ANNOSUS ON RESPONSE OF TRICHOCLADIUM ASPERUM TO INDOLEACETIC ACID.

INDOLEACETIC ACID CONCENTRATION		MILLIGRAMS DRY MYCELIUM								
		CONTROL	OPHIOBOLUS GRAMINIS				FOMES ANNOSUS			
			HOMOGENATE		SUPERNATANT		HOMOGENATE		SUPERNATANT	
			UNBOILED	BOILED	UNBOILED	BOILED	UNBOILED	BOILED	UNBOILED	BOILED
0		180.0 194.1 165.8	224.8 234.9 181.0	177.0 177.7 182.4	196.0 186.5 180.7	158.2 185.0 188.6	208.1 192.2 174.3	217.5 203.4 203.3	174.1 178.7 165.6	192.5 175.4 174.5
	Total	539.9	640.7	537.1	563.2	531.8	574.6	624.2	518.4	542.4
	Mean	180.9	213.6	179.0	187.7	177.3	191.5	208.1	172.8	180.8
50×10^{-5} M		192.6 182.1 190.0	169.4 200.4 189.4	189.8 201.8 194.2	182.0 205.3 188.2	188.3 180.3 171.2	154.3 92.3 96.0	185.1 203.4 199.7	153.2 154.9 122.6	182.3 196.5 204.5
	Total	565.6	559.2	585.8	576.4	539.8	342.6	588.2	430.7	583.3
	Mean	188.5	186.4	195.3	192.1	179.9	114.2	196.1	143.6	194.4

TABLE 32 (CONTINUED).

ANALYSIS OF VARIANCE.

SOURCE OF VARIATION.	D.F.	M.S.	F.	L.S.D. at 1%	L.S.D. at 5%
Treatments	17	1533.7	6.65	34.1	25.4
Error	36	230.6			
Total	53				

homogenate caused a significant decrease in growth in 50×10^{-5} M indoleacetic acid compared with the control. The differences produced by the homogenate of *O. graminis* and the supernatant of *F. annosus* were smaller but were significant at the 5% level of probability. The results are recorded in Table 32.

Thus it may be concluded that *O. graminis* and *F. annosus* are able to break down indoleacetic acid, while *T. viride* and *T. asperum* probably do so to a lesser degree. The products of this breakdown are toxic at least to *T. asperum*.

DISCUSSION AND CONCLUSIONS.

The results reported above indicate that satisfactory growth of *T. viride* and *T. asperum* occurred at concentrations of indoleacetic acid much above those at which growth of *O. graminis* and *F. annosus* is stopped. Whether this is a characteristic difference between parasitic and saprophytic fungi in general can only be determined by repeated tests with other representative strains and species. Considerable differences commonly occur between strains of the same species with regard to many physiological characteristics. As all degrees of parasitism and 'saprophytism' are known to exist among fungi, clear-cut distinctions are not to be expected. That a distinction was found between two parasites and two saprophytes is in itself of interest.

Enzyme preparations from higher plants which destroy indoleacetic acid have recently been the subject of considerable study (111, 131, 193, 223, 224). These authors usually refer to the enzyme as indoleacetic

acid oxidase, activity usually being followed manometrically by oxygen uptake. No direct evidence was obtained in the results reported here that oxidation of indoleacetic acid occurred, but the effect of 2,4-dichlorophenol in promoting its inhibitory activity is suggestive, for the latter substance has been shown to be an activator of indoleacetic acid oxidase in peas (82).

Apart from the report of Sequeira and Steeves (181) attributing leaf drop caused by Omphalia flavida to auxin inactivation, indoleacetic acid oxidase activity does not appear to have been studied in fungi. It is reasonable to expect that the higher plant will provide an environment in which indoleacetic acid will occur more consistently than in the soil. Relatively greater ability on the part of parasites to metabolize this substance, therefore, would not be surprising. That self-inhibition is the result of this ability is, however, paradoxical.

Although it is difficult to correlate concentrations of indoleacetic acid reported to be present in plant tissue with concentrations in liquid media, it is of interest that, calculated on a fresh weight basis, concentrations of indoleacetic acid of up to 100 milligrams per litre have been reported for plant tissues (120) and these concentrations would be inhibitory to O. graminis and F. annosus. Such concentrations are, however, probably exceptional and parasitic fungi in nature are unlikely to meet with concentrations that are inhibitory to them. It must be assumed that the breakdown of lower concentrations of indoleacetic acid does not result in the accumulation of products in sufficient amount to inhibit growth. The nature of the breakdown products from the action of indoleacetic acid oxidase preparations from higher plants has not yet

been determined, although Stutz (193) has demonstrated chromatographically that several substances are produced.

If high concentrations of indoleacetic acid are not commonly met with by parasitic fungi, their inhibition by these concentrations may have no significance for the parasitic mode of life. The ability to breakdown lower concentrations of indoleacetic acid, where inhibition does not develop, may be of some significance.

Although no satisfactory explanation of the effects of indoleacetic acid in higher plants have been forthcoming, of late attention has been given to the effect of indoleacetic acid on the pectin constituents of the cell wall (31, 80, 81, 152). These reports do not appear to be in agreement as to what such effects are, but it is of interest to note that Ordin et al. (152) have found indoleacetic acid to promote methyl esterification of the pectin constituents in the cell wall. Jansen and MacDonnell (108) demonstrated that demethylation of pectin was an essential preliminary for optimal activity of polygalacturonase in pectin breakdown. If the breakdown of pectin is important in the invasion of cells by parasitic fungi, as has been suggested by the work of Brown (e.g. 30), then in living host cells inactivation of indoleacetic acid may be first necessary for satisfactory activity of the fungus enzymes involved in pectin degradation. Of interest in this connection is a recent study made by Shaw and Hawkins (182) of the levels of indoleacetic acid and indoleacetic acid oxidase activity in uninfected cereal leaves and in cereal leaves infected with rust or mildew. They found that the level of indoleacetic acid first declined and then increased in infected leaves, while indoleacetic acid oxidase activity showed a very marked increase

immediately following infection, followed by a decline which was rapid in susceptible hosts and slower in resistant hosts. It would certainly be of interest to determine if the oxidase activity was of host or parasite origin. If the oxidase activity proves to be of fungal origin then some support for the results reported here may be found. In apparent contradiction of these findings Pilet (159) concludes from studies of _Uromyces pisi_ on _Euphorbia cyparissias_ that an inhibitor of indoleacetic acid oxidase is produced by the fungus.

It is certainly too early to attempt to formulate any theory incorporating the results of the investigations reported here. The contradictory nature of results obtained from studies of indoleacetic acid in higher plants alone would suggest that any explanation of these findings is likely to be complex.

CONCLUSIONS.

The investigations reported here were initiated for the purpose of determining differences in the physiology of two parasitic and two saprophytic fungi. Three physiological aspects have been studied and the main conclusions to be drawn are as follows.

In their response to various antibiotics the saprophytes appeared generally more tolerant than the parasites. This observation accords well with the relatively greater ability of the saprophytes to live freely in the soil. As a generalization it should, however, be qualified. In some cases the parasites showed proportionately less inhibition than _T. viride_, (e.g. by griseofulvin), and frequently demonstrated considerable adaptation. This adaptation is of interest but its nature is not understood. It is not known, for example, whether enzymic breakdown of the antibiotic was involved with the synthesis of an adaptive enzyme as a preliminary. But these qualifications do indicate that possibly under certain conditions the presence of antibiotics might not in itself be sufficient to limit saprophytic growth by these fungi.

It was also noted that the response of _T. viride_ and _T. asperum_ to antibiotics was not the same. _T. viride_ tended to be inhibited to a greater extent than _T. asperum_, but when inhibited, frequently showed adaptation. _T. asperum_, on the other hand, was little influenced by the antibiotic concentrations used, but where it was inhibited gave little indication of adaptation.

Considerable differences were found between the four fungi with

regard to their nutritional requirements.

The growth rates of the two parasites were similar and lay in between those of the saprophytes. T. viride had a very rapid rate of spread on solid media, which together with its ready ability to sporulate, must confer a considerable advantage in colonizing fresh and possibly ephemeral substrates in the soil. T. asperum, on the other hand, grew much more slowly than either of the parasites, but its growth was dense and produced a high mycelial weight on liquid cultures - both factors which indicate efficient use of food materials. Possibly the disadvantage which T. asperum has with regard to growth rate is somewhat compensated for by its greater tolerance to antibiotics. It should be noted, too, that T. asperum also produced an abundance of spores.

The study of the influence of temperature and pH on growth of the fungi also revealed interesting differences. Thus the temperature and pH ranges of F. annosus were much more limited than those of the other fungi, and this is probably of no disadvantage to a fungus which resides for long periods in the woody tissues of tree roots and trunks where the environmental conditions remain relatively constant for long periods. The greater pH and temperature range of O. graminis is, on the other hand, probably essential for its satisfactory development on and in the somewhat shorter lived roots of cereals. The observation that at lower temperatures it may grow better than T. viride, a potential soil antagonist, may be important in disease development in the field (Ludwig and Henry (129)). The tolerance of T. viride and T. asperum to reasonably wide temperature and pH ranges suggests that these fungi would show considerable adaptation to soil conditions varying in these characteristics.

Both of the parasitic fungi showed requirements for vitamins and preferences for complex nitrogen sources. Although these may not be exclusively characteristics of parasites, it is certainly true that such requirements are more likely to be met in the host plant than in the soil. The complementary observations that the saprophytes grew satisfactorily on simple synthetic media without supplements of this kind suggests that this is a difference of considerable importance.

Under the conditions of adequate vitamin and nitrogen nutrition the parasites and the saprophytes made good growth on many of the carbohydrates studied. Many individual differences were apparent and it can be seen that together with certain combinations of some of the other factors studied, the availability of different carbohydrates in an environment could be a controlling influence in selection. Such differences in utilization could form the basis of some very interesting metabolic studies. Marked differences between the parasites and saprophytes as groups were not too evident, although the clear-cut distinction drawn by the ability of the parasites to make good growth on insoluble cellulose was certainly of interest. The importance of cellulose as a cell wall constituent in higher plants requires no emphasis, and its potential importance as a substrate for invading parasitic fungi is obvious.

Striking differences were found in the response of the fungi studied to indoleacetic acid. That the level at which inhibition occurred was similar in _O. graminis_ and _F. annosus_ and much lower than that for the saprophytes is felt to be of considerable interest. This inhibition was shown to be correlated with a relatively greater ability on the part of the parasites, and particularly _F. annosus_, to carry out an enzymic

breakdown of indoleacetic acid. The products of this enzyme reaction were found to be inhibitive at least to T. asperum, suggesting that the relative tolerance of the saprophytes to indoleacetic acid inhibition probably resides in their inability to convert this substance to some inhibitory breakdown product. A possible significance of these findings for the parasitic ability of O. graminis and F. annosus has already been discussed, but it should be emphasized here that they were probably the clearest distinctions between the two pairs of fungi revealed in these investigations.

Further elaboration of the studies of indoleacetic acid breakdown would be of considerable value, especially as this activity does not appear to have been investigated in fungi.

A completely satisfactory conclusion to these studies would be reached only when sufficient information was obtained to explain why one fungus must be a saprophyte and another a parasite. This ideal has most certainly not been attained here, but the findings do give some indication of the suitability of each fungus for the habitat in which it is found. Thus it is easy to appreciate why T. viride appears to be such an important soil saprophyte. Tolerance to antibiotics (and the ability to produce at least two such substances (23)); high growth rate; production of spores in abundance; wide pH range; ability to grow on simple nitrogenous compounds without an exogenous supply of vitamins; and ability to utilize a variety of carbohydrates; these are all characteristics which would be expected to make for saprophytic success. Most of these characteristics were exhibited also by T. asperum. Similarly, the results of these investigations indicate why O. graminis

and F. annosus are not likely to be successful soil inhabitants and are suggestive as to why the host plant is a more favourable habitat for these fungi.

The value of further study can hardly be overstated, for it can only be from a thorough understanding of the environmental requirements of fungi that a knowledge of parasitism, and of saprophytism too, can be gained, and only then can a thoroughly intelligent approach to the control of disease be made.

SUMMARY.

Studies were undertaken to compare certain aspects of the physiology of representative saprophytic and parasitic soil inhabiting fungi. Four fungi were studied in particular, two saprophytes, Trichoderma viride Pers. ex Fries, and Trichocladium asperum Harz, and two parasites, Ophiobolus graminis Sacc. and Fomes annosus Fries.

Comparisons were made with regard to tolerance to antibiotics, nutritional behaviour, and response to plant growth substances.

The effects of five antifungal antibiotics, namely, acti-dione, gliotoxin, griseofulvin, patulin and trichothecin, were studied.

1. Acti-dione inhibited all four fungi to some extent. T. asperum was least affected; T. viride was initially strongly inhibited by concentrations of 10 p.p.m. and higher, but showed good adaptation. Both O. graminis and F. annosus were very strongly inhibited by acti-dione. The former did not grow at 5 p.p.m. and the latter failed to grow above 10 p.p.m.
2. Gliotoxin inhibited growth of F. annosus more strongly than that of the other fungi, which were fairly similar in their response to this antibiotic.
3. T. viride appeared proportionately more susceptible to griseofulvin inhibition than any of the other three fungi. O. graminis was fairly tolerant to this antibiotic and showed good adaptation. F. annosus was more tolerant than T. viride at the lower concentrations.
4. Patulin, even at 100 p.p.m., reduced growth of T. asperum only slightly. Greater inhibition occurred with T. viride, while O. graminis and F. annosus

were strongly inhibited. The last two fungi, however, showed good adaptation at the lower concentrations.

5. Trichothecin was initially quite inhibitory to *T. viride*, but good recovery occurred. The responses of *T. asperum* and *O. graminis* were rather similar but *F. annosus* was strongly inhibited by this antibiotic even at 1 p.p.m.

It was concluded from the study of antibotics that the saprophytes were generally more tolerant than the parasites.

The various aspects of the nutrition of the four fungi studied included growth rates, temperature and pH responses, requirements for vitamins and nitrogenous compounds, and the utilization of various carbohydrates.

6. *T. viride* was observed to have a very high growth rate while that of *T. asperum* was comparatively slow. The growth rates of *O. graminis* and *F. annosus* were similar and lay between those of the two saprophytes.

7. Growth of the four fungi was observed at 10°C., 15°C., 20°C., 25°C., 30°C. and 35°C. Only *T. viride* grew at 35°C. but here growth was soon arrested. The optimum for *T. viride* was 25-30°C.; it grew poorly at 15°C. and 10°C. *T. asperum* grew very slightly at 30°C.; the optimum was 20-25°C. and it grew comparatively well at 10°C. *O. graminis* made a little growth at 30°C.; the optimum was 20-25°C. and comparatively good growth occurred at 10°C. The optimum temperature for *F. annosus* was 25°C.; only a trace of growth occurred at 15°C. and 30°C. was the upper temperature limit.

8. The two saprophytes appeared rather more tolerant of low pH than the parasites. *O. graminis*, *T. viride* and *T. asperum* made good growth at

the higher pH values used, but growth of F. annosus was much reduced at pH 7.0. The optimum for F. annosus appeared to be pH 5.0.

9. T. viride and T. asperum grew as well with potassium nitrate as sole nitrogen source as with other forms of nitrogen. This was not true of O. graminis and F. annosus which grew best on organic nitrogen.

10. Neither T. viride nor T. asperum required an exogenous supply of vitamins. F. annosus required thiamine and O. graminis required thiamine and biotin for growth on a synthetic medium containing either inorganic or organic nitrogen.

11. The four fungi showed considerable differences in their ability to utilize representative pentose and hexose monosaccharides, disaccharides and polysaccharides. Compared with D-glucose the following were found to be poor carbon sources: for T. viride, D-galactose, L-arabinose, D-arabinose, maltose, sucrose, inulin, pectin and cellulose; for T. asperum, D-galactose, L-arabinose, D-arabinose, D-ribose, lactose, melibiose, pectin and cellulose; for O. graminis, D-galactose, D-arabinose, D-ribose, melibiose, inulin and pectin; for F. annosus, D-galactose, L-arabinose, D-arabinose, D-ribose, cellobiose and inulin.

The results of the nutritional studies indicated that the requirements of T. viride and T. asperum would probably be satisfied in the soil, while those of O. graminis and F. annosus would be fulfilled optimally in their hosts.

The effects of indoleacetic acid, 2,4-dichlorophenoxyacetic acid and indolebutyric acid, on the growth of the four fungi used, were studied.

12. Indoleacetic acid inhibited O. graminis and F. annosus at low concentrations. T. viride and T. asperum were not appreciably inhibited at concentrations up to 100 x 10^{-5} M. No consistent stimulation of growth

was recorded.

13. Additional fungi were also included in these studies and the response, to indoleacetic acid, of those which were saprophytes was generally similar to that of T. viride and T. asperum.

14. The inhibition by indoleacetic acid was enhanced by 2,4-dichlorophenol at 10^{-4} M.

15. 2,4 -Dichlorophenoxyacetic acid had no appreciable effect on the growth of the fungi on solid media at concentrations up to 250 x 10^{-5} M. Indolebutyric acid inhibited the growth of O. graminis and F. annosus at 100 and 250 x 10^{-5} M to a greater extent than that of T. viride or T. asperum.

16. F. annosus was shown to be highly active in breaking down indoleacetic acid.

17. The breakdown products produced by homogenates of O. graminis and F. annosus were shown to be toxic to T. asperum.

From the studies with indoleacetic acid it was concluded that the response of O. graminis and F. annosus was fundamentally different from that of T. viride and T. asperum. It was considered that this difference might have some significance for the parasitic mode of life of the first two fungi.

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